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THÈSE

En vue de l'obtention du DOCTORAT DE L'UNIVERSITÉ DE TOULOUSE

Délivré par l'Université Toulouse 3 - Paul Sabatier

Présentée et soutenue par
Thomas GENAIS

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**Caractérisation de la fonction et du mode d'action d'Enok, une
Histone Acétyltransférase de type MOZ, dans le contrôle de la
prolifération et de la différenciation des cellules sanguines de
drosophile**

Ecole doctorale : **BSB - Biologie, Santé, Biotechnologies**

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Summaries

Résumé

L'hématopoïèse est un processus très finement régulé qui mène à la formation de chaque cellule sanguine d'un organisme. Chez les mammifères il existe un nombre important de types cellulaires qui participent à l'établissement des mécanismes de défense du corps. Toutes ces cellules proviennent de la différenciation terminale d'une cellule unique appelée Cellule Souche Hématopoïétique (CSH) qui, par le biais de différenciations successives donnant naissance à des progéniteurs de plus en plus spécifiés, va permettre l'établissement normal de tous les types de cellules sanguines. Les CSH sont parmi les cellules les plus finement contrôlées de l'organisme, car en effet une dérégulation de leur fonctionnement normal (prolifération excessive, différenciation prématurée...) peut entraîner de graves conséquences, à savoir des maladies du sang appelées leucémies.

De nombreux facteurs moléculaires sont impliqués dans la régulation des CSH, et certains d'entre eux sont les cibles de mutations ou réarrangements chromosomiques à l'origine de leucémies, tels que le facteur de transcription RUNX1 et la Lysine Acétyl-Transférase (KAT) Monocytic Leukemia Zinc-Finger (MOZ).

Chez la Drosophile, les cellules sanguines sont apparentées au lignage myéloïde des mammifères, et les acteurs moléculaires contrôlant leur formation sont très conservés. Ainsi, les cellules à cristaux (CC), qui sont les homologues fonctionnels des mégakaryocytes, sont formées suite à l'action conjointe de l'homologue de RUNX1, Lozengue (Lz) et de GATA1, Serpent (Srp). Un criblage pan génomique mené par mon équipe d'accueil visant à trouver des modulateurs de l'activité transcriptionnelle de Lz et Srp, a permis de d'identifier le gène *enoki mushroom* (*enok*) comme étant un régulateur négatif de cette activité. Enok est l'homologue chez la Drosophile de MOZ, et une étude préliminaire de son rôle *in vivo* a pu mettre en évidence une fonction essentielle dans le développement des CC, qui disparaissent quasiment en totalité dans un contexte mutant pour *enok*.

L'objectif de ma thèse a été de comprendre les mécanismes par lesquels Enok régule la formation des CC chez la larve de Drosophile.

Les CC sont générées au stade larvaire à partir de la transdifférenciation de macrophages après activation par la voie Notch, et l'initiation de Lz. Au contraire de données publiées récemment par un autre groupe, j'ai démontré que Lz est requis et suffisant pour induire l'expression de l'effecteur de la voie Hippo Yorkie, et non l'inverse. De plus, grâce à des expériences de perte de fonction et de sauvetage phénotypique, j'ai montré qu'Enok est requis pour l'expression de Lz de façon autonome cellulaire dans les précurseurs de CC.

Chez les mammifères, MOZ est connu pour faire partie d'une tétrade d'acétylation, et j'ai montré qu'ici seul un de ses partenaires est requis, le facteur de type BRPF Br140. Pour approfondir l'étude de la fonction d'Enok, j'ai généré grâce au système CRISPR/Cas9 un allèle catalytiquement inactif d'*enok*. J'ai ainsi montré que cette fonction n'est absolument pas requise durant la différenciation des CC.

Enfin, j'ai montré qu'Enok permet la régulation de l'expression de Lz en se fixant sur un enhancer dans le troisième intron du gène. De façon intéressante, sa délétion provoque le même phénotype de perte de CC qu'une perte de fonction d'*enok*, montrant qu'il est requis pour l'expression de Lz dans le système hématopoïétique.

Ces résultats mettent en valeur un nouveau mode d'action d'Enok dans l'hématopoïèse chez la Drosophile. De façon intéressante, une étude récente propose qu'Enok et Br140 se fixent sur la chromatine pour réguler l'expression de gènes cibles. Mes résultats sur une fonction non-catalytique d'Enok viennent enrichir ce modèle, qui montre un mode d'action peu exploré dans le domaine. Dans le futur, ce travail pourrait ouvrir des portes sur une meilleure compréhension des fonctions normales et pathologiques de MOZ chez les mammifères.

Abstract

Hematopoiesis is a very tightly regulated process leading to the normal production of every blood cells in an organism. In mammals there is a lot of cell types that participate to the establishment of the defense mechanisms of the body. All those cells come from the terminal differentiation of a single cell called Hematopoietic Stem Cell (HSC) which will differentiate to give rise to committed progenitors that will eventually differentiate all blood cell types. HSCs are among the most controlled cells in the organism. Indeed, deregulation of their normal function (excessive proliferation, premature differentiation, ...) can be at the onset of severe blood pathologies called leukemias.

Several molecular factors are involved in HSC regulation, and some of them, like RUNX1 and the Monocytic Leukemia Zinc-Finger protein (MOZ), are targets of mutations or chromosomal rearrangements that lead to a leukemic transformation.

In *Drosophila*, blood cells share functional homology with the mammalian myeloid lineage, and the molecular actors controlling their formation are well conserved. Indeed, differentiation of crystal cells (CC), which have similar functions than megakaryocytes, occurs following the interaction of RUNX1 homolog, Lozenge (Lz) and GATA1 Serpent (Srp). With the aim at identifying regulators of Srp/Lz transcriptional activity, a genome-wide screen led by my team allowed the identification of *enoki mushroom* (*enok*) as a strong negative regulator of this activity. Enok is the homolog of MOZ in *Drosophila*, and a preliminary study *in vivo* showed that Enok is essential for CC development during the larval stage, as CC almost completely disappear in *enok* loss of function context.

The objective of my PhD was to understand the mechanisms by which Enok regulates CC formation in the *Drosophila* larvae.

During the larval stage, CC are generated by the transdifferentiation of macrophages, after their activation by the Notch signaling pathway and onset of Lz expression. In contrast to data published by another group, I demonstrated that Lz is required and sufficient to initiate the expression of the Hippo signaling pathway effector Yorkie, and not the contrary. Furthermore, using loss of function and rescue experiments, I showed that Enok is absolutely required cell autonomously in CC precursors for Lz expression but not for the proper Notch signaling which appears normal in an *enok* mutant context.

In mammals, MOZ is known to be a part of an acetylation complex, and I showed that only one of its members, the BRPF factor Br140, is required alongside with Enok during CC differentiation. To go further into the characterization of Enok mode of action, I generated a catalytically inactive *enok* mutant using the CRISPR/Cas9 system and demonstrated that this function is completely dispensable for CC differentiation in the larvae.

Finally, I show that Enok regulates the maintenance of *lz* expression by binding to an enhancer located in its third intron. Hence, deletion of this enhancer provokes the same loss of CC phenotype as what is observed after *enok* loss of function showing that this enhancer is required for *lz* expression in the hematopoietic system.

These results highlight a new mode of action of Enok during *Drosophila* hematopoiesis. Interestingly, a recent study proposed that Enok and Br140 bind chromatin together in order to regulate gene expression. Therefore, my results on a non-catalytic activity of the *drosophila* Enok brings new insights into this new model, which uncovers a mode of action not much explored in the field. In the future, my work should open new avenues and might allow a better understanding of MOZ normal functions in mammals and thus give rise to more efficient ways to treat MOZ-associated pathologies.

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I. Preamble

Hematopoiesis is the process that leads to the normal production of every mature blood cell type in an organism; by definition, maintenance of its homeostasis is crucial throughout development, as hematopoietic deregulation has dramatic consequences on the organism. Indeed, blood disorders are even nowadays among the deadliest pathologies. In order to maintain a normal blood content, the hematopoietic process is tightly regulated, and this regulation is extensively studied in biological research with the aim of understanding the molecular mechanisms that are deregulated in pathologies and of finding new efficient ways to cure them.

All mammalian blood cells come from the differentiation of Hematopoietic Stem Cells (HSCs). These HSCs have the essential property of being able to self-renew indefinitely; it has been shown that they can regenerate the entire hematopoietic system of irradiated mouse recipients through several successive graft experiments (Jacobson et al. 1951a; Spangrude, Heimfeld, et Weissman 1988). HSCs differentiate asymmetrically and give rise to more and more committed progenitors that ultimately generate all blood cell types.

HSC discovery led many groups to pursue the study of those cells during normal and pathologic hematopoiesis. Several models emerged over the years to study the tight regulation controlling the formation of every blood cell lineage, such as vertebrate models like mouse and zebrafish. However, the strong conservation of molecular factors controlling hematopoiesis across evolution shed light on the *Drosophila* model, which has been widely used for over fifteen years for the study of both normal and pathological hematopoiesis.

I will describe in a first part how mammalian blood cells are formed under normal and pathological conditions and which molecular actors are involved during those processes. In a second part, I will summarize what is known about *Drosophila* blood cell formation and show why it has emerged as an invaluable model to study normal and pathologic hematopoiesis. Finally, I will describe the discovery of *enoki mushroom* and how it was linked to a function in hematopoiesis, which is the basis of my thesis work.

II. Introduction

A. Normal Hematopoiesis in vertebrates and mammals

1. Blood cell types and functions

Mammalian hematopoiesis is characterized by a great number of cell types that are formed; ten different cell types and several subtypes are produced (reviewed in (Orkin et Zon 2008; Weiskopf 2016); (Figure 1). These highly specialized cells of the myeloid and lymphoid lineages ensure a large panel of functions.

a) Myeloid lineage

Myeloid cells are responsible for innate immunity, which allows a rapid and direct defense of the organism against invading pathogens, wound healing and transport of gases. Cells involved in the innate immunity are found in many organisms, and innate immunity is the major defense system in insects and plants.

- **Granulocytes** are distributed in three types: neutrophils, eosinophils and basophils that are categorized based on their ability to absorb neutral, acid or basic eosin-derived dyes. Neutrophils are phagocytes and can exocytose granules, thereby reducing inflammation. Eosinophils are responsible for the elimination of parasites, using cytotoxic granules, and basophils produce histamine during inflammatory and allergic reactions.
- **Macrophages** arise from the terminal differentiation of monocytes and are responsible for phagocytosis. They eliminate apoptotic or necrotic cells, and also cancer cells that express specific membrane markers. They also have a defense function during innate immunity, as they phagocytize pathogens like bacteria. Macrophages are furthermore found as resident cells in several adult tissues where they self-maintain (Hashimoto et al. 2013); in addition, they participate in maintaining the steady state of tissues. Indeed, macrophages are able to activate BMP signaling in enteric neurons in order to control gastrointestinal motility (Muller et al. 2014). It also has been shown that macrophages

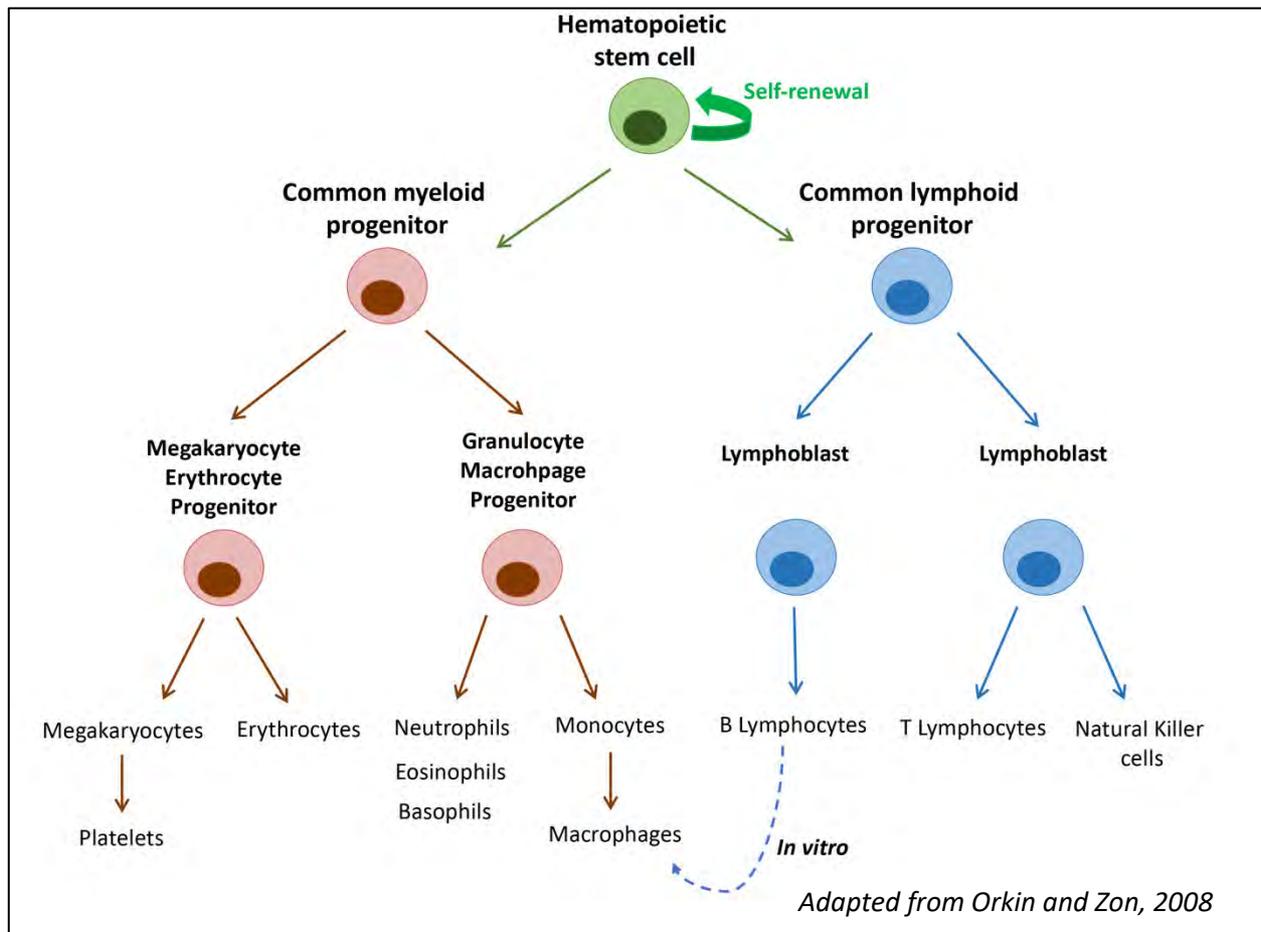


Figure 1: Representation of the mammalian hematopoietic tree

At the top of the tree, hematopoietic stem cells are responsible for the formation of every type of blood cell. Asymmetric divisions allow self-renewal and generate progenitors of the myeloid and lymphoid lineages. These progenitors further differentiate into more committed progenitors that will eventually give rise to all mature blood cells. The blue dashed line indicates the ability of B lymphocytes to transdifferentiate into macrophages. (Adapted from Orkin and Zon, 2008)

protect tissue against inflammatory response by inhibiting neutrophil recruitment (Uderhardt et al. 2019).

- **Megakaryocytes** are responsible for the production of platelets that are released in circulation after megakaryocyte fragmentation and, upon injury, they aggregate to allow wound healing by promoting coagulation.
- **Erythrocytes** are the most abundant blood cell type; they are enucleated cells mostly responsible for the transport of oxygen throughout the organism during embryonic and adult life. They have other functions such as regulating blood viscosity and maintaining the shear stress necessary for vascular development and remodeling (Baron 2013; Lucitti et al. 2007).

b) Lymphoid lineage

Lymphoid cells are responsible for adaptive immunity, a second, highly specific, wave of defense against invading pathogens. Adaptive immunity arose in gnathostomes, as all jawed vertebrates possess lymphocyte receptors.

- **B lymphocytes** are key effectors of the adaptive response. They are activated by T lymphocytes (see below) at the onset of the response to initiate the production of antibodies specific to the pathogen. A subset of B lymphocytes subsequently becomes memory B cells and allows an efficient response, if the organism has to fight the same pathogen later in life.
- **T lymphocytes** are classified in 3 main groups of cells: (i) T helper cells (positive for the CD4 marker) that are activated after the presentation of an antigen by Antigen Presenting Cells; the activated T cell then interacts with a B lymphocyte to trigger specific antibody production; (ii) cytotoxic T lymphocytes (positive for the CD8 marker) that directly eliminate the pathogen after their activation and (iii) memory T lymphocytes that have a long lifespan and participate in long-term immunity.
- **Natural Killer cells**, thanks to their cytotoxic granules, are responsible for direct destruction of cancer cells, viral-infected cells and foreign bodies.

Adult definitive blood cells all come from HSCs, localized in the bone marrow. This environment is a niche allowing HSCs to proliferate and differentiate normally (reviewed in (Asada, Takeishi, et Frenette 2017)). However, these HSCs themselves emerge after successive events taking place during embryonic development that I will describe below.

2. Blood cell formation

Blood cell formation is a process that initiates during early embryonic stages. Indeed, during its development, the embryo has to be supplied in oxygen and may also have to be able to fight off some pathogens that would be a threat to its survival. In order to meet those needs, three successive hematopoietic waves take place during development (Figure 2).

a) First hematopoietic wave and primitive hematopoiesis

The first hematopoietic wave consists of a transient myelopoiesis that takes place inside blood islands of the yolk sac during early embryogenesis (reviewed in Lcaud et Kouskoff 2017). Absence of primitive hematopoiesis in the mouse embryo, notably after loss of function of GATA-1, which is a major hematopoietic transcription factor, leads to early embryonic lethality presumably due to the absence of erythrocytes (Fujiwara et al. 1996); this phenotype underlines the importance of early hematopoiesis during development.

This first embryonic wave gives rise to primitive myeloid cells (erythrocytes, megakaryocytes and macrophages) that will migrate to the embryo proper. Some studies suggested that endothelial cells and primitive blood cells in blood islands of the yolk sac arise from a common progenitor called hemangioblast, but although it seems to be conserved from fly to mouse (Mandal, Banerjee, et Hartenstein 2004; Vogeli et al. 2006; Huber et al. 2004), this has not yet been demonstrated in humans and is a matter of debate. In mouse, primitive erythrocytes come from erythroblasts produced in the blood islands that enter the bloodstream, continue to divide (Bethlenfalvay et Block 1970) and finally differentiate into enucleated erythrocytes that express both embryonic and adult globins (Kingsley 2004, 2006;

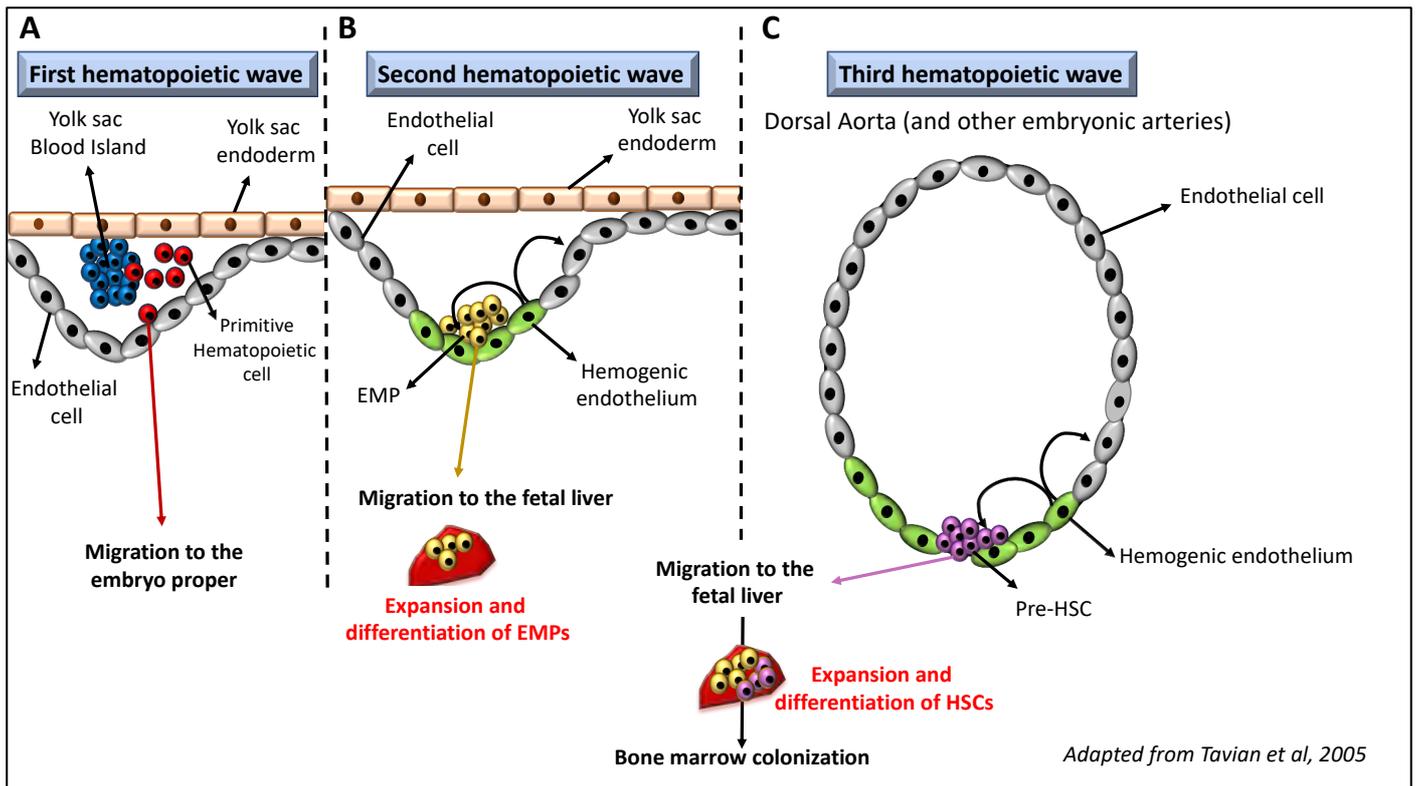


Figure 2: Embryonic waves of hematopoiesis in mammals

A. During the first wave, primitive hematopoietic cells (in red) arise from cells inside blood islands (in blue) in the yolk sac. They migrate to the embryo proper, where they differentiate into primitive erythrocytes, macrophages and megakaryocytes. **B.** During the second wave, the first definitive blood cells arise. These cells are Erythromyeloid Myeloid Progenitors (EMPs, in yellow); they originate from the hemogenic endothelium (in green) of the yolk sac through the Endothelial to Hematopoietic Transition process and migrate to the fetal liver, where they proliferate and differentiate. **C.** The third wave gives rise to the hematopoietic stem cells (HSCs). They emerge from the hemogenic endothelium of major arteries in the embryo, and in particular in the dorsal aorta. The emerging pre-HSCs will then, like EMPs, migrate to the fetal liver to expand and differentiate. At the end of embryogenesis, newly formed hematopoietic cells will migrate again to colonize the bone marrow niche. (*Adapted from Tavian et al, 2005*)

Qiu et al. 2008). It also has been shown that in the yolk sac, primitive megakaryocytes emerge from bipotent progenitors called megakaryocyte/erythroid progenitors (MEPs) that can give rise to both primitive lineages (Tober et al. 2007). These megakaryocytes participate in the formation of embryonic platelets that circulate in the embryo. Primitive macrophages in the yolk sac have two origins: a first population is composed of maternally-derived macrophages that do not express all adult macrophages markers, and a second population comes from monopotent progenitors that differentiate into macrophages exhibiting definitive adult features (Bertrand 2005).

Hematopoietic cells formed during the first embryonic wave are transient and thus are replaced later on during embryogenesis by definitive cells with adult features. Consequently, they are not found in post-natal stages and are not involved in definitive hematopoiesis. However, it has recently been shown that primitive macrophages play an essential role in colonizing hematopoietic organs, notably by remodeling the extra cellular matrix (Travnickova et al. 2015).

b) Second hematopoietic wave and emergence of the first definitive blood cells

In addition, the second hematopoietic wave occurs in the extra embryonic yolk sac, with the emergence of a myeloid progenitor called the Erythromyeloid Progenitor (EMP; reviewed in (Frame, McGrath, et Palis 2013). EMPs emerge from the hemogenic endothelium of the yolk sac (Frame et al. 2016) through a process called Endothelium to Hematopoietic Transition (EHT). EMPs migrate rapidly through the new circulatory system to colonize the fetal liver (Palis 1999), where they proliferate and differentiate into mature macrophages, megakaryocytes and erythrocytes. These cells are considered part of definitive hematopoiesis, since they have the same characteristics as cells arising later on from the differentiation of HSCs (Bertrand 2005). The cells produced by the differentiation of EMPs are mostly transient, but some of them can be found in the adult organism, where some resident macrophages in the adult tissues derive from EMPs (Schulz et al. 2012; Gomez Perdiguero et al. 2015).

Although EMPs lack any lymphoid potential, it has been shown that lymphocytes are present in the yolk sac during early embryogenesis, anterior to the emergence of HSCs (Yoshimoto et al. 2011, 2012), and that they come from independent lymphoid progenitors.

c) Third hematopoietic wave, HSC emergence and bone marrow niche

The third and last embryonic wave gives rise to the HSCs. They were first shown to emerge in the aorta-gonad-mesonephros (AGM) region of the mouse embryo, through the process of EHT from hemogenic endothelium in the dorsal aorta (Medvinsky et Dzierzak 1996; de Bruijn 2000; Tavian et Peault 2005). After this discovery it was shown that HSCs can arise from hemogenic endothelium in several regions of the embryo, including the yolk sac, the umbilical and vitelline arteries, and the placenta (de Bruijn 2000; Gekas et al. 2005; Chen et al. 2009). Then, newly formed hematopoietic cells called pre-HSCs delaminate from the endothelium and enter the circulation to colonize the fetal liver, probably *via* umbilical vessels. Once in the liver, they rapidly expand and mature to become fully competent (repopulating) HSCs (Kieusseian et al. 2012). They can then yield committed progenitors that in turn give rise to the complete hematopoietic system (Mikkola 2006). Finally, around the time of birth, HSCs colonize the bone marrow, where their proliferation and differentiation properties are tightly controlled by the microenvironment provided by the niche during adult life.

The bone marrow compartment constitutes a very complex microenvironment (reviewed in Morrison et Scadden 2014; Asada, Takeishi, et Frenette 2017) to protect HSCs against pathological transformation. In this niche HSCs remain mostly quiescent, with a limited rate of self-renewal activity that ensures continuous production of new hematopoietic cells in normal proportions (Akunuru et Geiger 2016).

d) A particular case of blood cell formation: the transdifferentiation process

Even though all cells come from the differentiation of HSCs, it has been shown that *in vitro*, B lymphocytes can be reprogrammed by a process called transdifferentiation (H. Xie et al. 2004); during this process committed B cells are induced to become another type of committed cell, macrophages, without passing through a progenitor step. They simultaneously dedifferentiate from their original identity and start to express macrophages markers, meaning that at some point these cells express genes of both lineages (Jopling, Boue, et Belmonte 2011; Cieślak-Pobuda et al. 2017). Although rarely observed, this phenomenon does happen in mammals, yet it has been more thoroughly characterized in *Drosophila* (see section C-2b).

The fine equilibrium between proliferation and differentiation of HSCs can sometimes be unbalanced (notably by the mutation of key molecular actors such as transcription factors of epigenetic regulators), and this deregulation leads to the development of severe pathologies such as leukemia, which I will describe below.

B. Pathological hematopoiesis and its major actors

1. Leukemias: multifaceted malignant blood disorders

There are four major classified types of leukemia, depending on which cell lineage is affected and on the invasiveness of the disease: chronic, acute myeloid or lymphoid leukemia.

Chronic leukemia impacts 1 out of 100 000 persons per year in France, and this condition can persist for several years. In this disease, progenitor cells are not affected but do not produce mature cells able to fight off infections efficiently. With time, immature cells replace normal cells in the bone marrow niche, which is deleterious for the organism, because it completely impairs the ability of the immune system to respond to infection.

Acute leukemia has an incidence of 4 out of 100 000 cases per year in France and has a much poorer prognosis, as it is very invasive. Contrary to chronic leukemia, acute leukemia

affects progenitor cells: it is characterized by the induction of immature blood cells and abnormal proliferation that rapidly inhibits production of differentiated cells in the niche.

Classically, leukemias appear as the consequence of somatic mutations accumulated during life. Those mutations have been classified into two categories (De Kouchkovsky et Abdul-Hay 2016). Class I mutations affect genes implicated in proliferative pathways (such as c-Kit and STAT3; The Cancer Genome Atlas Research Network 2013; Yamada et Kawauchi 2013), and class II mutations affect genes implicated in normal blood cell formation (such as NMP1 and CEBPA; The Cancer Genome Atlas Research Network 2013); according to the classical double hit model of leukemogenesis, class I and II mutations have to occur concomitantly to trigger the pathology. However, over the past few years, a third type of mutation has emerged, which corresponds to mutations targeting epigenetic regulators (such as DNMT3A and TET2; Patel et al. 2012) involved both in proliferation and differentiation. This double hit model is based on punctual mutations, but it does not take into account bigger genomic instabilities such as chromosomal rearrangements (translocations, duplications...), which in some cases can be sufficient to initiate a leukemic state.

Chromosomal rearrangements can affect many genes, but among them some events are more represented. This is the case for rearrangements affecting the locus encoding the major hematopoietic factor RUNX1/AML1 (Miyoshi et Ohki 1991), or loci encoding the MOZ and MORF epigenetic enzymes of the MYST family (Borrow et al. 1996). I will describe their functions in both normal and pathological hematopoiesis in the following sections.

2. The highly conserved family of RUNX transcription factors and its links to leukemia

The RUNX family is composed of Core Binding Factor (CBF) transcription factors strongly conserved across evolution of bilaterian metazoans (Rennert et al. 2003). Its founding member, the product of the *runt* gene, was described for the first time in *Drosophila melanogaster* in 1980 and was shown later on to be essential for embryonic segmentation (Gergen et Wieschaus 1986). In *Drosophila*, there are four RUNX genes: *runt*, *RUNXA*, *RUNXB* and *lozenge (lz)*, and in mammals only three: RUNX1, RUNX2, RUNX3 (Figure 3). These

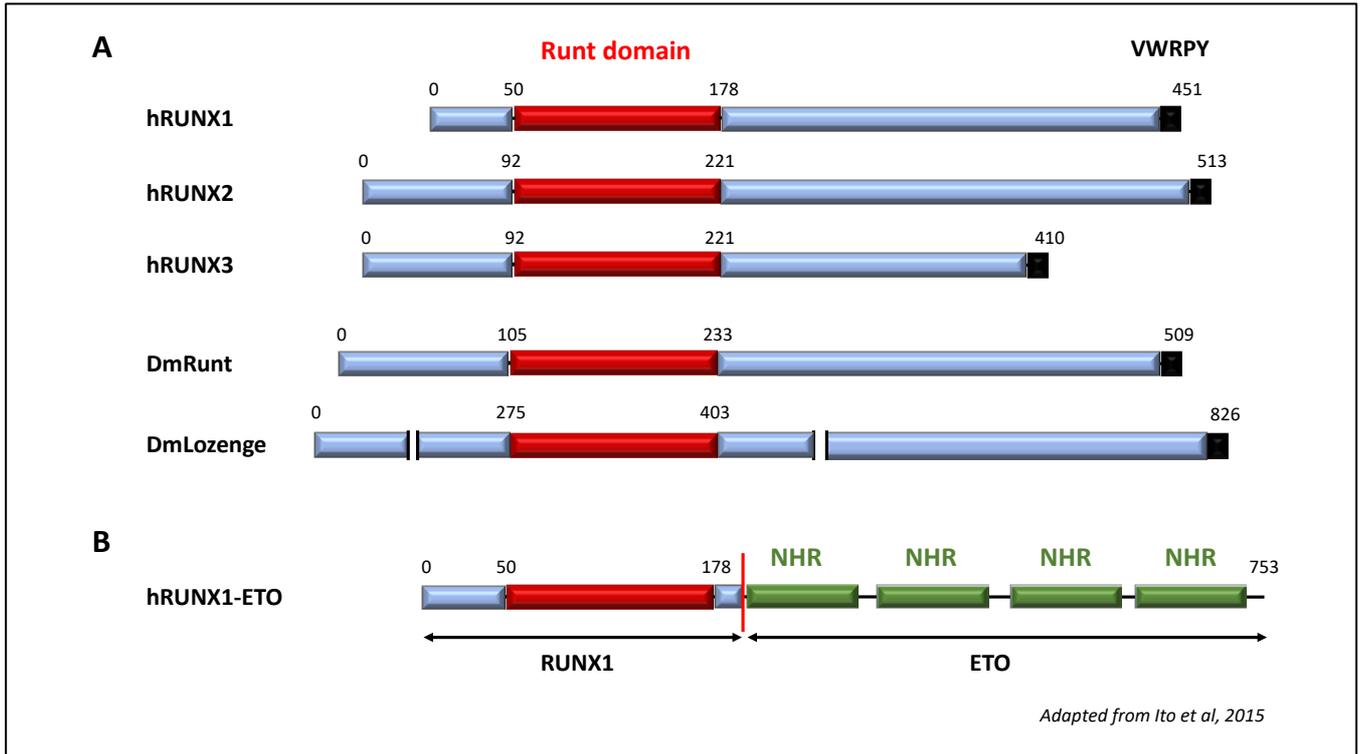


Figure 3: RUNX transcription factors

A. Schematic representation of the three human RUNX proteins, along with the Drosophila Runt and Lozenge RUNX factors. Runt is the founding member of the RUNX family; Lozenge is a RUNX gene ortholog, and the only identified RUNX protein involved Drosophila hematopoiesis. All RUNXs possess a highly conserved Runt domain responsible for DNA binding and a VWRPY motif involved in interactions with co-repressors. **B.** Schematic representation of the leucemogenic fusion protein RUNX1-ETO: the full-length Runt domain of RUNX1 is fused to the ETO protein, which contains four Nery Homology Region (NRH) domains that are mostly known to allow interaction with repressors. (Adapted from Ito et al, 2015)

transcription factors are involved in many processes like hematopoiesis, neurogenesis and bone development (Ito 2008), as well as in various diseases (Chuang, Ito, et Ito 2013; C.-C. Sun et al. 2019). They all contain a highly conserved runt domain, which allows both DNA binding on a specific TGYGGTY recognition motif and heterodimerization with their partner CBF β (Adya, Castilla, et Liu 2000; Ito, Bae, et Chuang 2015). Furthermore, they possess a C-terminal VWRPY motif responsible for the interaction with co-repressors, such as the Groucho/TLE family (Aronson et al. 1997; Levanon et al. 1998).

In human, RUNX1 was first identified as the target of chromosomal translocations associated to the development of acute myeloid leukemia (AML; Miyoshi et Ohki 1991; Erickson et Gao 1992). It is one of the most frequently mutated loci, as RUNX1 is altered in 26% of AML cases, either by a single point mutation or by chromosomal rearrangements (Haferlach et al. 2014). A RUNX1 mutation in AML is almost always linked with poor prognosis. This association of RUNX1 mutations with the development of severe pathologies is indicative of a prominent role of RUNX1 during normal hematopoiesis (see below). The second mammalian RUNX transcription factor, RUNX2, was identified on the basis of its sequence homology with RUNX1 (Kamachi et al. 1990) and is notably involved in the ossification process (Takarada et al. 2016). Finally, RUNX3 was identified in 1994 (Levanon et al. 1994) and has been shown to be a tumor suppressor gene involved in many cancers (notably colorectal cancer; Bae et Choi 2004). Furthermore, it has been shown that in human gastric carcinoma cells, RUNX3 is responsible for the expression of miR-182, which in turn inhibits cancer growth (Yu et al. 2017). Together, RUNX1 and RUNX3 are involved in breast cancer, where they have a protective effect against epithelial to mesenchyme transition (Kulkarni et al. 2018). RUNX3 not only has roles in pathologies, but is also involved in normal neural development (Appel et al. 2016) and in hematopoiesis, where it cooperates with RUNX1 for the differentiation of several subtypes of T lymphocytes (Woolf et al. 2003, 3; Li et al. 2012, 3). Furthermore, RUNX3 has a role of its own; it modifies the chromatin landscape during Cytotoxic T lymphocyte formation (D. Wang et al. 2018).

It thus appears that RUNX genes are key regulators of normal and pathological development, and that their regulation is essential to maintain homeostasis of several tissues, including the hematopoietic tissue, where RUNX1 plays a crucial role in the development of AML.

a) RUNX1 in Acute Myeloid Leukemia

- RUNX1-ETO at the onset of AML

RUNX1 is the target of several chromosomal rearrangements that lead to the induction of AML, among which the most common is the t(8;21) translocation that fuses *RUNX1* with the *Eight Twenty-One* gene (*ETO*) (Erickson et al. 1992; S. C. Bae et al. 1993). Little is known about ETO function, but it is mostly involved in gene repression, since it can recruit several co-repressors (Salat et al. 2008; Issay Kitabayashi et al. 1998) through its nerved homology (NRH) domains (Y. Liu et al. 2006). This translocation is the most common in *de novo* AML (Grimwade et al. 2010) and affects mostly elder people. It provokes an inhibition of wild-type RUNX1 and leads to a block in myeloid differentiation, associated with a high and abnormal self-renewal rate of progenitors (Okuda et al. 1998), which gives the AML phenotype. It was shown recently that the RUNX1-ETO (also called RUNX1-RUNX1T1) fusion protein interacts with several factors such as Lmo2 and the ETS factor PU.1, to inhibit the expression of differentiation genes and activate the expression of stem cell genes (Ptasinska et al. 2014; X.-J. Sun et al. 2013). Furthermore, RUNX1-ETO drives changes in the chromatin landscape that modify the epigenome and facilitate leukemic functions (Loke et al. 2017). However, it has been proposed that the t(8;21) translocation is not able to trigger leukemogenesis on its own, and that it has to be combined with a secondary mutation (Grisolano et al. 2003; Kelly et al. 2002). In about 20-40 % of cases, a mutation in the c-KIT gene (a crucial factor in HSC development) was concomitantly identified (W. Xie et al. 2019; Y.-Y. Wang et al. 2005).

- Other leukemogenic mutations of RUNX1

Another translocation affecting RUNX1 is the t(3;21) leading to the *RUNX1-EVI1* fusion gene (Nucifora et al. 1994; Mitani et al. 1994). This translocation is mostly found as a secondary hit in already established blood pathologies, like chronic myeloid leukemia (Nukina et al. 2014). It leads to the same phenotype as RUNX1-ETO, even if the molecular targets are not the same (Loke et al. 2017). In addition to chromosomal rearrangements, several point mutations in the *RUNX1* gene were identified, all involved in the initiation of AML (Gaidzik et al. 2011).

Therefore, RUNX1 appears to be a major target in pathologies affecting hematopoiesis, and its function during the malignant process is extensively studied. But the fact that its deregulation leads to severe disorders, is an indication pointing towards its important role during normal hematopoiesis.

b) RUNX1 during normal hematopoiesis

RUNX1 is expressed in the progenitors of the two definitive embryonic waves (second and third waves), as well as in some primitive macrophages (T. North et al. 1999) and in adult HSCs (Ng et al. 2010; Nottingham et al. 2007). It is a major regulator of both embryonic and adult hematopoiesis; indeed, RUNX1 is crucial for EMP and HSC emergence during EHT (Chen et al. 2009, 1; T. E. North et al. 2002; Yzaguirre et al. 2018; Liakhovitskaia et al. 2014, 41). RUNX1 mutant mouse embryos are very pale and lack any fetal liver hematopoiesis (Okuda et al. 1996, 1), phenotypes that lead to early lethality due to severe hemorrhages. This evidence underlines its importance during the establishment of hematopoietic cells, and in particular for HSC development and fetal liver colonization. Consistently, primitive hematopoiesis of the first embryonic wave does not seem to be affected in the RUNX1 mutant, showing that this wave is RUNX1 independent. It is not clear in the literature if RUNX1 is involved in HSC maintenance, but several studies show that it is expressed in the bone marrow compartment (Ng et al. 2010; Nottingham et al. 2007). Finally, it is known that during adult hematopoiesis, RUNX1 is involved in myeloid differentiation and in particular for megakaryocytes formation (Draper et al. 2016). RUNX1 is also responsible for B and T lymphoid development (Chi et al. 2018; Woolf et al. 2003, 3; Li et al. 2012, 3; Taniuchi et al. 2002).

RUNX1 expression and function are regulated and mediated by several factors that play a role upstream, downstream, or in collaboration with RUNX1, in order to generate every blood cell not only in the embryo but also in the adult. Here I describe RUNX1's main partners.

c) RUNX1 partners and regulators during normal hematopoiesis

- Notch is a major regulator of RUNX1 expression

The Notch pathway is crucial during development and can be involved in several processes, like proliferation, asymmetrical cell division (and thus differentiation), or even cell death. Notch is a membrane receptor composed of two parts: an intracellular domain (NICD) responsible for the transcriptional activity of the pathway and an extracellular domain (NECD) responsible for the interaction with its ligands Delta/Serrate/Jagged (DSL). Once the ligand/receptor interaction occurs, the Notch receptor is cleaved multiple times, a first time (S2 cleavage) by the ADAM metalloproteinase and a second time (S3 cleavage) by the γ -secretase complex. This separates NECD from NICD, and the released intracellular part will then relocalize to the nucleus. There, it interacts with a complex composed of the Notch pathway transcription effector called CSL (CBF1/RBPJ, Suppressor of Hairless(Su(H)), Lag-1), Mastermind (Mam) and other coactivators to regulate their target genes (Figure 4; reviewed in Bray 2006; Kovall et al. 2017). In mammals, there are four Notch receptors: Notch1-4.

As described above, HSCs arise from the hemogenic endothelium of the major embryonic arteries, and in particular in the dorsal aorta. Its hemogenic potential is controlled and maintained by Notch signaling from the interaction between the ligand Jag1 and the receptor Notch1 (Kumano et al. 2003, 2; À. Robert-Moreno et al. 2008, 1; Clarke et al. 2013).

Notch1 directly regulates the expression of GATA2, a marker of hematopoietic cells within the hemogenic endothelium required for HSC development (A. Robert-Moreno 2005; de Pater et al. 2013, 2), and is at the origin of the onset of RUNX1 expression in hematopoietic cells (Nakagawa et al. 2006). Then, Notch expression is downregulated in the cells undergoing EHT (Richard et al. 2013). Although Notch1 is required during the process of HSC formation, it has been shown that RUNX1 allows the emergence of EMPs from the yolk sac hemogenic endothelium independently of Notch1 (Hadland 2004; Bertrand et al. 2010).

- GATA transcription factors are known RUNX interactors

The GATA family is composed of transcription factors conserved across evolution. They are characterized by the presence of one or two zinc finger domains that allow DNA binding

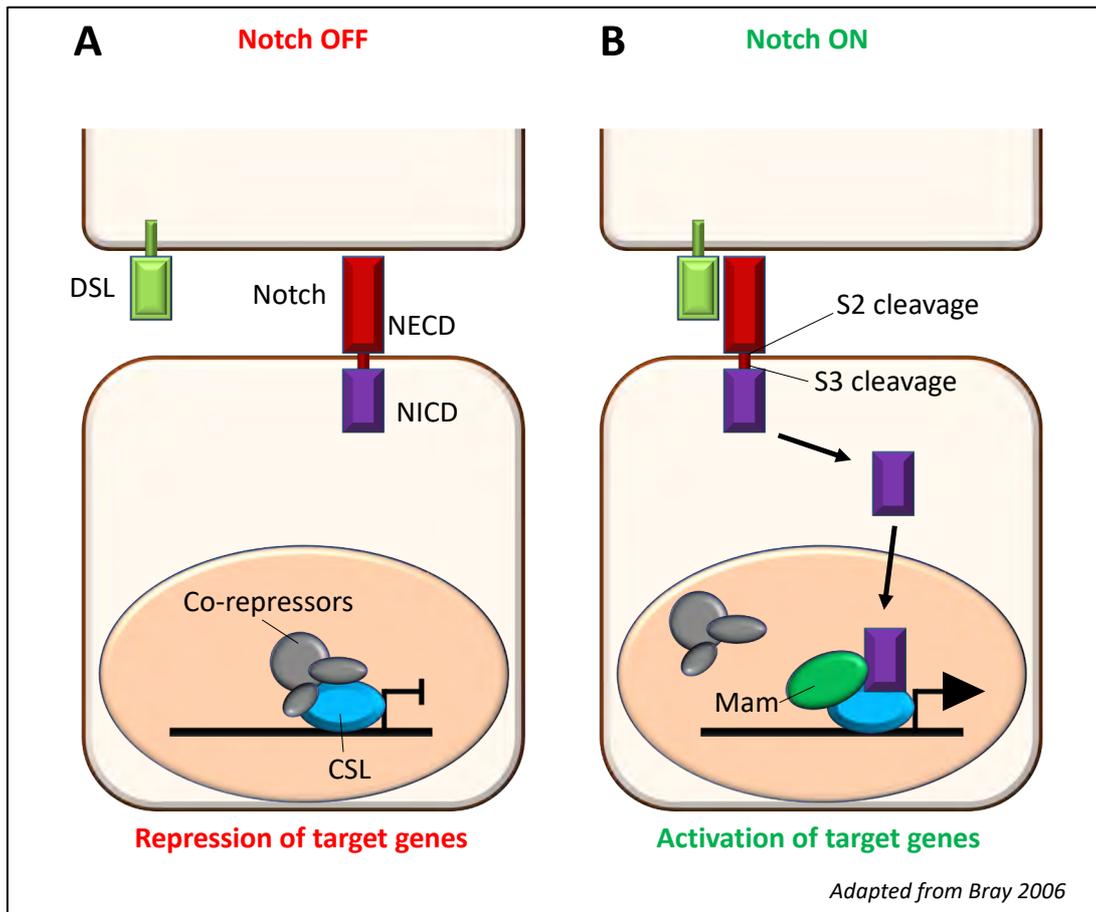


Figure 4: Overview of the Notch signaling pathway.

A. When the pathway is inactive, the CSL transcription factor is bound by co-repressors and Notch target genes are repressed. **B.** Notch pathway is activated by the interaction between one Delta Serrate Ligand (DSL) and the Notch ExtraCellular Domain (NECD) of the receptor. Upon interaction, the receptor undergoes a series of cleavages (S2 and S3) that lead to the translocation of the Notch IntraCellular Domain (NICD) into the nucleus. There, it interacts with its CBF1/RBPJ, Suppressor of Hairless (Su(H)), Lag-1 (CSL, blue) partner and with Mastermind (Mam, green) to replace co-repressors and activate transcription of target genes. (Adapted from Bray 2006)

or interaction with their cofactors Friend Of GATA (FOG). In mammals there are six GATA factors (GATA1-6), but only three of them (GATA1-3) are required during the hematopoietic process. GATA1 is a major actor of the hematopoietic development, as it is required for erythrocyte formation (described above, Fujiwara et al. 1996, 1) and megakaryocyte differentiation (Chang et al. 2002; Iwasaki et al. 2003, 1). GATA2 is absolutely required for HSC emergence and survival in the embryo (de Pater et al. 2013, 2; Ling et al. 2004, 2; Tsai et al. 1994). Initially, GATA3 was only shown to be required for the differentiation of T-helper-type-2 cells (G. R. Lee, Fields, et Flavell 2001; Ho, Tai, et Pai 2009, 3). However, it has been shown that GATA3 is expressed in lymphoid progenitors, where it is crucial for the formation of the T cell lineage (Rothenberg 2013), and some recent findings suggested that it also plays a role during HSC emergence in the embryo (Zaidan et Ottersbach 2018).

RUNX1 is required for megakaryocyte differentiation during definitive myelopoiesis (Draper et al. 2016), and it has been shown that GATA1 cooperates with RUNX1 in this process (Elagib 2003; Goldfarb 2009; Pencovich et al. 2011; Tijssen et al. 2011). It is interesting to note that Elagib *et al* show that the fusion protein RUNX1-ETO inhibits GATA1 during megakaryocytic differentiation in cell culture, which they propose to be a mechanism involved in the blocking of myeloid differentiation during AML development.

Furthermore, RUNX1 and GATA2 are both present in HSCs in the bone marrow but have never been shown to interact with each other. However, they cooperate during HSC emergence in the embryo (Wilson et al. 2010), and it has recently been shown that they are involved in a complex regulating the expression of the *SET* gene (which is a potent Inhibitor of Protein Phosphatase 2A) in two AML cell lines (HL-60 and HEL cells; Pippa et al. 2017).

- [CBF \$\beta\$, RUNX heterodimerization partner](#)

CBF β , a RUNX1 heterodimerization partner seems to be involved during the hematopoietic process, because a loss of function of this gene in the mouse embryo leads to the same phenotypes as a RUNX1 mutant (Niki et al. 1997). It is likely that all the roles played by RUNX factors during hematopoiesis are dependent on CBF β . Indeed, its loss of function specifically in the adult, has broader phenotypes than the ones of RUNX1 alone, indicating that CBF β interacts with other partners, which may be RUNX factors during this process.

- Monocytic Leukemia Zinc-Finger protein (MOZ)

MOZ is a Lysine Acetyltransferase (KAT) involved during hematopoiesis at several levels, that I will develop later in more detail. It was shown that in cultured myeloid mouse cells, MOZ was part of the RUNX1 complex and required for its transcriptional activity, therefore participating in myeloid differentiation (I. Kitabayashi 2001, 1; Yoshida et Kitabayashi 2008, 1). The authors propose that MOZ acts independently of its catalytic activity in this process, but this hypothesis has never been developed further. They also demonstrate that MOZ is able to acetylate RUNX1 *in vitro*, but this modification was never identified *in vivo*. Another study showed that MOZ and RUNX1 cooperate for the transcription of the Macrophage Inflammatory Protein 1 α (MIP-1 α) gene in human Jurkat T-cells (Bristow 2003), providing a new example of MOZ/RUNX1 interaction during hematopoiesis.

The epigenetic regulator MOZ and its paralog MOZ-Related Factor (MORF) are themselves common targets of chromosomal rearrangements leading to the induction of AML, and I will describe their role in normal and pathological hematopoiesis in the next part.

3. MOZ/MORF and the MYST family of Lysine Acetyl-Transferases

The MYST family is named after its founding members: MOZ, Yfb2, Sas2 and Tip60, and is a very conserved family from yeast to human (Figure 5). In mammals there are five members, MOZ/MORF, Tip60, Male absent of the First (MOF) and Human acetylase Binding to ORC1 (HBO1, reviewed in (Yang 2004; X.-J. Sun et al. 2015). KATs of the MYST family were first shown to acetylate histone tails, and by doing so, to participate in the remodeling of the chromatin landscape (Grant 2001; Goll 2002). However, studies over these last years yielded increasing evidence that they have a much broader range of substrates than just histones (reviewed in Sapountzi et Côté 2011). KATs of the MYST family are involved in a variety of pathologies, but I will focus here on the role of MOZ and its paralog MORF in hematopoietic disorders.

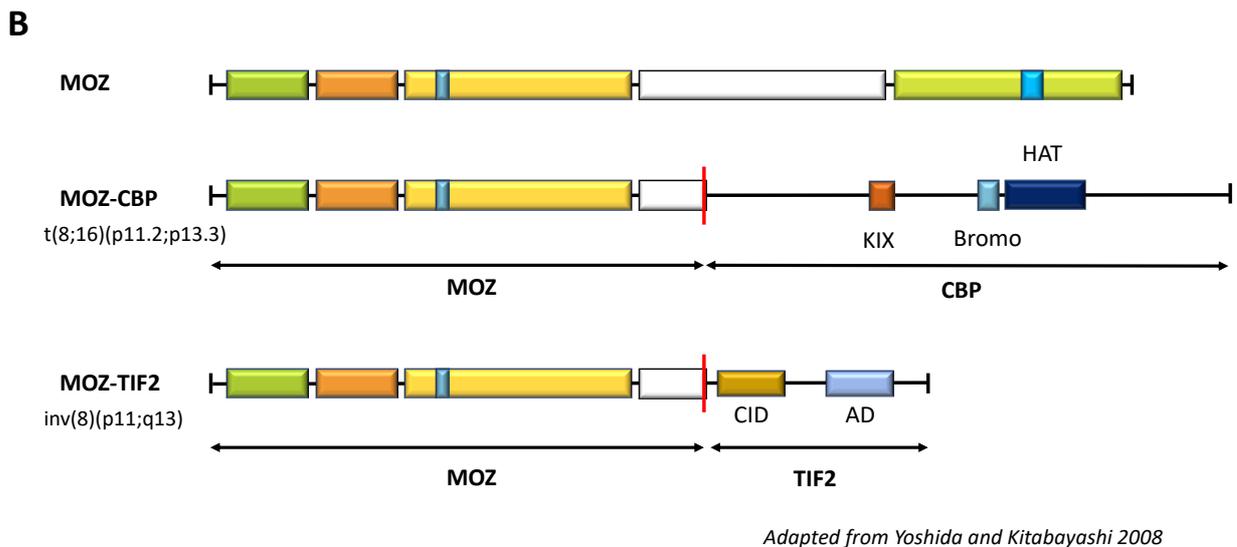
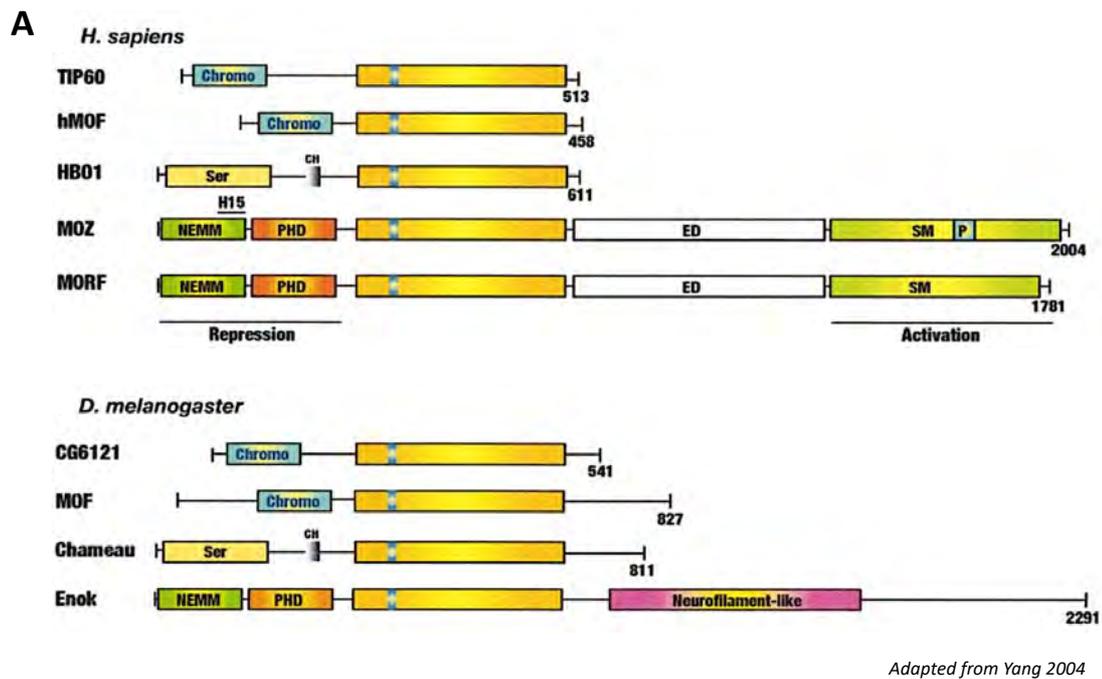


Figure 5: MYST family of acetyl transferases

A. Schematic representation of human and *Drosophila* MYST proteins. All of them possess a conserved MYST domain with catalytic activity. **B.** Schematic representation of MOZ and the main fusion proteins involved in leukemia. In the MOZ-CBP fusion, the N-terminal part of MOZ comprises a functional MYST domain fused to the C-terminal part of CBP. In the MOZ-TIF2 fusion, the same breakpoint in MOZ is fused to the CBP Interacting Domain (CID) domain of TIF2, which is responsible for CBP binding. (Adapted from Yang 2004 ; Yoshida and Kitabayashi 2008)

a) MOZ and MORF in AML

The name MOZ comes from Monocytic leukemia Zinc-finger protein, because it was first identified as a target of chromosomal translocations responsible for the development of AML. These rearrangements involve in particular the CREB-binding Protein (CBP) (Borrow et al. 1996; Chaffanet et al. 2000; Crowley et al. 2005) and TIF-2 (inv(8)(p11q13); Figure 5; Carapeti et al. 1999). MOZ protein contains several important domains that have been characterized and linked to specific functions. The MYST domain bears the acetylation catalytic activity and contains a C2HC zinc-finger involved in DNA binding (Holbert et al. 2007). This conserved MYST domain is also required for the interaction with MOZ partners Bromodomain-PHD finger proteins (BRPF) 1 2 and 3 (Ullah et al. 2008). The C-terminal domain of MOZ is called SM for Serine/Methionine-rich domain and is involved in transcription activation. This domain is also involved in the interaction with p53 (Susumu Rokudai et al. 2009; S. Rokudai et al. 2013; Tham et al. 2015), which is a transcription factor involved in cell cycle regulation, autophagy and apoptosis (Sabapathy et Lane 2019). Finally the N-terminal part of MOZ, is composed of the NEMM domain (for N-terminal of Enok MOZ/MORF; these proteins are the only members of the MYST family that contain this N-terminal part) and a double PHD (Plant Homeodomain) finger, which contains a recognition domain for histones and is involved in transcription inhibition (Dreveny et al. 2014; Xiong et al. 2016).

MOZ and CBP are both important factors for HSC maintenance and quiescence (Katsumoto 2006; Thomas 2006; Rebel et al. 2002; Bilal N. Sheikh et al. 2017), and TIF2 (Transcriptional Intermediary Factor 2) is a direct partner of CBP (Demarest et al. 2002).

The leukemogenic MOZ-CBP fusion protein contains the N-terminal part of MOZ with its intact MYST domain, and almost the entire CBP protein (Chan et al. 2007). As a result, the recognition repertoire of CBP is aberrantly acetylated by MOZ and leads to the proliferation of leukemic progenitors and to the AML phenotype. Similarly, MOZ-TIF2 has been shown to recruit CBP via the CID interaction domain of TIF-2, which results in CBP inhibition and leads to leukemic development (Deguchi et al. 2003; Kindle et al. 2005). It also has been shown that MOZ-TIF2 represses senescence in AML stem cells and thus leads to their expansion (Largeot et al. 2016). It is noteworthy that MORF, a paralog of MOZ, is also involved in a chromosomal

translocation t(10;16) with CBP, associated to the development of AML (Panagopoulos 2001), suggesting that MORF could play a role during normal blood cell formation.

In addition to its involvement in leukemia, MOZ has also been linked to the development of other pathologies, such as intellectual disability (Tham et al. 2015) and esophageal adenocarcinoma (Dulak et al. 2013), and its transcriptional deregulation leads to metastasis of medulloblastoma and colorectal cancer (Wu et al. 2012; Mohammadi et al. 2018). It is interesting to note that in a recent study, Baell *et al* identified a new inhibitor of MOZ that is sufficient to stop cancer growth in a model of mouse lymphoma, by inducing senescence in cancer cells (Baell et al. 2018).

As for RUNX1, MOZ is an important target of leukemogenic mutations and rearrangements, which prompted many research groups to study its role during normal hematopoietic development.

b) MOZ during normal hematopoiesis

MOZ was first shown to display a KAT activity on histone tails (N. Champagne, Pelletier, et Yang 2001) and has since been studied mainly considering this property. Indeed, in a murine model carrying a point mutation in the catalytic site of MOZ, Perez-campo *et al* showed that MOZ-mediated acetylation is required for proliferation and expansion of hematopoietic progenitors (F. M. Perez-Campo et al. 2009). Later, the same group showed that MOZ catalytic activity is required to prevent HSCs from entering into replicative senescence, thus promoting their self-renewal capacity (Flor M. Perez-Campo et al. 2014). MOZ is also required in for their maintenance, since its loss of function in the embryo leads to the absence of hematopoietic progenitors and adult-repopulating cells in the fetal liver (Katsumoto 2006; Thomas 2006). Furthermore, specific MOZ loss of function in the adult bone marrow compartment leads to a rapid loss of HSCs (B. N. Sheikh et al. 2016), as measured by their loss of ability to reconstitute the hematopoietic system in an irradiated mouse recipient. Finally, it was shown that MOZ acts in collaboration with the polycomb group protein BMI1 to maintain HSCs in a quiescent state (Bilal N. Sheikh et al. 2017), ensuring their maintenance over time.

In addition to its function in HSCs, MOZ is required for myeloid lineage and lymphoid B-cells formation: it is a coactivator of RUNX1, in particular for MIP-1 α expression, and is required for normal macrophage and B-cell development (Jiang et al. 2019; Katsumoto 2006; Good-Jacobson et al. 2014).

As I previously mentioned, MOZ acts as a transcriptional coactivator of RUNX1, in a KAT-independent manner (I. Kitabayashi 2001). There are consistent differences between the phenotypes of mice completely deprived of any MOZ activity (Katsumoto 2006) (embryonic lethality and strong depletion of the hematopoietic system) and phenotypes of mice specifically deprived of its catalytic function (F. M. Perez-Campo et al. 2009) (viable with reduced progenitor proliferation, but no effect on differentiation). These observations strongly support the hypothesis that MOZ has other non-catalytic functions during both development and hematopoiesis.

It has been shown that MOZ has broad functions during development, in addition to its roles in hematopoiesis. Indeed, it is an important player during embryonic development, as it regulates histone acetylation of HOX genes during mouse body segmentation (Voss et al. 2009; Bilal N. Sheikh et al. 2015).

c) MOZ and the ING5 acetylation complex

KATs of the MYST family generally act as members of acetylation complexes (reviewed in Lee et Workman 2007). It has been shown that MOZ is a part of such an acetylation complex, working within the ING5 complex, along with Esa1-Associated-Factor 6 (EAF6), Inhibitor of Growth 5 (ING5) and BRPF1 2 or 3 (Figure 6; Ullah et al. 2008) to specifically modify histone H3. In this complex, MOZ interacts directly with BRPF factors via its MYST domain, and BRPF itself interacts directly with EAF6 and ING5 (Ullah et al. 2008). BRPF proteins are epigenetic readers that recognize specific histone modifications in order to bind chromatin and allow proper modification of the chromatin landscape. BRPF1 has been shown to recognize in particular H3K14ac, therefore directing MOZ to its substrates (Poplawski et al. 2014). In addition, BRPF1 shares common functions with MOZ; indeed, BRPF1 participates in the expression of HOX genes during development in zebrafish (Hibiya et al. 2009), and it is also

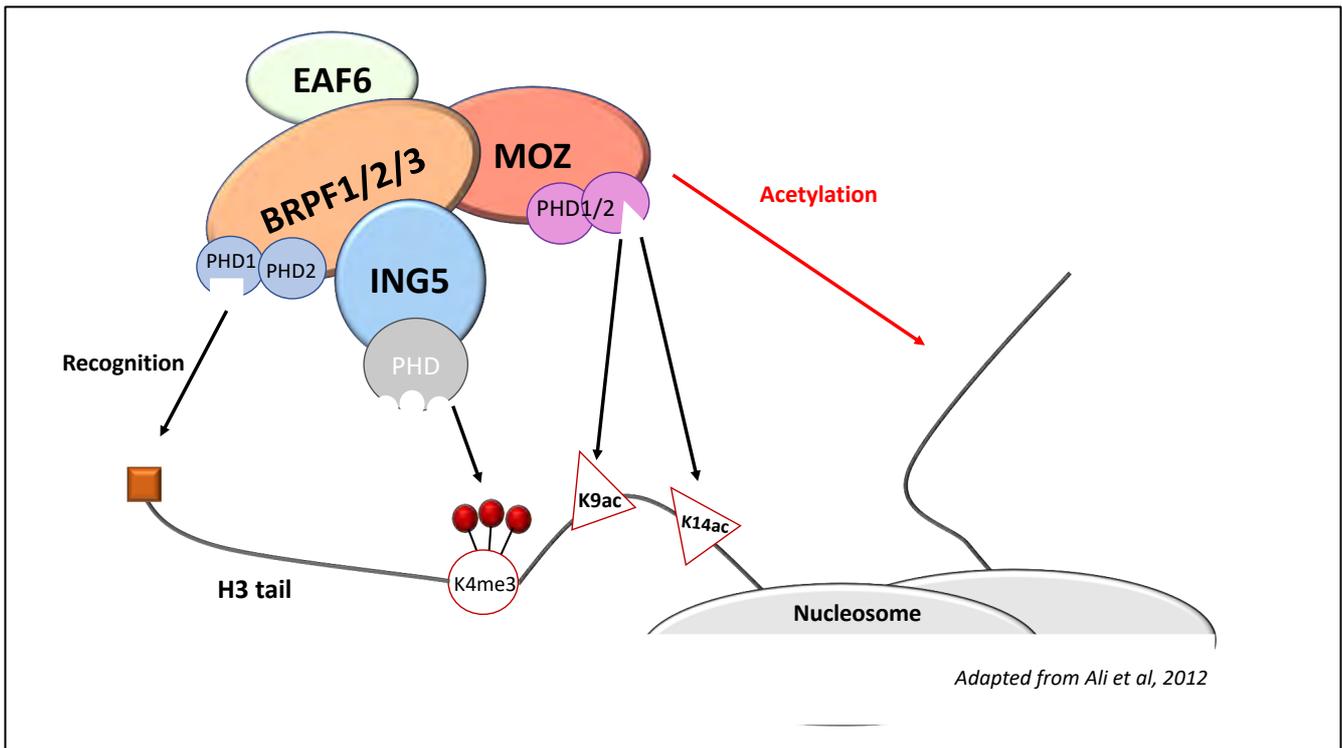


Figure 6: The ING5 acetylation complexes

MOZ, which is the catalytic subunit of the complex, directly interacts with the BRPF factors. The other members, EAF6 and ING5, also specifically interact with BRPF but not directly with MOZ. Together, the complex is able to recognize precise chromatin motifs that allow acetylation of a specific repertoire. The BRPF factor recognizes the histone H3 tail *via* its PHD fingers, ING5 recognizes trimethylation of H3K4, and MOZ recognizes acetylated H3K9 and H3K14. In turn, MOZ will acetylate another H3 tail on lysine 9 or 14. (Adapted from Ali et al 2012)

essential for the normal establishment of fetal liver HSC (You et al. 2016). ING5 is a Tip60 cofactor (N. Liu et al. 2013) and is important for cell proliferation in human cultured cells (Linzen et al. 2015). Furthermore, it has been shown to recognize histone H3 modification H3K4me3 via its PHD domains (K. S. Champagne et al. 2008, 3; Ormazza et al. 2019, 4). The function of EAF6 has not been described so far.

In summary, the ING5 complex provides an environment that enables a specific recruitment of MOZ on its target repertoire, allowing MOZ to specifically acetylate its substrates (Ali et al. 2012).

Mammalian hematopoiesis is a very complex system. Indeed, the need to produce numerous cell types in physiological proportions requires a high level of regulation and therefore a lot of molecular interplay. Moreover, the classical model for hematopoiesis in mammals is the mouse model, which is complicated to manipulate. Mice possess 20 chromosomes and achieve puberty at 2 months of age, which makes it a poor genetic model. However, the hematopoietic process is strongly conserved in vertebrates, both at the developmental and genetic levels. All the molecular factors described above (Notch, RUNX, GATA, MOZ...) are highly conserved across evolution, and in addition to vertebrate models, *Drosophila melanogaster* has proven these last fifteen years to be a very powerful model to study the molecular mechanisms controlling the development of innate immunity.

C. *Drosophila* as a model to study hematopoiesis

1. Generalities on the model

Drosophila, which means “who likes dew”, also called the fruit fly, is an animal model used in many research fields, and in particular in genetic and developmental studies. In labs, the subfamily *melanogaster* (“black belly”) is widely used.

Thomas Hunt Morgan, a zoologist, was the first to use this model in his lab (later called the Fly Room) in 1910 and received the Nobel Prize for his discovery concerning the role of

chromosomes during the process of heredity. He and his group identified many genes, which are still used today as genetic markers for experiments, like the *white* gene that is responsible for the red color of the eye.

This animal model is particularly easy to use in genetic studies, because it only has four pairs of chromosomes, three pairs of autosomes and one pair of sexual chromosomes. In addition, meiotic recombination does not occur in males, which is of particular interest when doing genetic experiments. Moreover, *Drosophila* generation time is quite short, around 10 days at 25°C, which makes it easy to design *in vivo* experiments.

Finally, it is a highly relevant model concerning medical research, because it has been shown that 75 % of disease-related genes have a sequence similarity in the fly, and that 50 % of fly proteins have mammalian homologs (Reiter 2001).

The fruit fly has a life cycle with four stages: embryo, larva, pupa and adult (Figure 8A). The embryonic stage lasts for twenty-four hours, ending with the emergence of the larva. This larval stage is itself composed of three stages called L1, L2 and L3 and lasts for five days. Then, the larva produces an ecdysone (a steroid hormone) signal that leads to a “wandering” behavior and finally to pupariation. The pupal stage also lasts for five days, and during this stage the individual undergoes the metamorphosis process, in which tissues are remodeled and give rise to the adult morphology. Finally, the adult fly emerges from the pupa and begins to mate eight to twelve hours thereafter.

Many developmental processes are conserved throughout evolution like neurogenesis, organ formation, stem cell biology, and of course hematopoiesis. *Drosophila* is an ideal model to study these processes and many discoveries in *Drosophila* participated in our understanding of mammalian (and particularly human) development. Here, I will describe the process of hematopoiesis in flies and how it can be used as a model for this process in normal and pathological situations.

2. *Drosophila* hematopoiesis

Drosophila is an organism that possesses an open circulatory system in which circulates a liquid called the hemolymph. In larvae, the dorsal vessel (or aorta), which is positioned dorsally in the antero-posterior axis, ends with the “heart” in the posterior part. This heart beats to generate a flow of hemolymph that circulates in all the tissues and enables blood cells to migrate. In *Drosophila*, blood cells share functional similarities with the mammalian myeloid lineage. In 1957, Rizki characterized three cell types: the plasmatocytes, the crystal cells and the lamellocytes (Figure 7; M. T. M. Rizki 1957) which are called hemocytes.

a) *Drosophila* blood cell types

- Plasmatocytes

Plasmatocytes are the most abundant cells, as they represent about 95 % of all blood cells in *Drosophila*. They are phagocytes with functional homology to macrophages. They are crucial players of innate immunity in *Drosophila*, since they are responsible for the elimination of bacteria (Charroux et Royet 2009; Defaye et al. 2009; Nehme et al. 2011) or virally-infected cells (Nainu et al. 2015). Plasmatocytes are also partly responsible for the production of antimicrobial peptides after bacterial infection (Irving et al. 2005).

Moreover, they play an essential role during development, as they phagocytose apoptotic bodies both in the embryo and in the pupa, particularly after tissue remodeling during metamorphosis (Defaye et al. 2009; Wood et Martin 2017; Lanot et al. 2001; Regan et al. 2013). Furthermore, plasmatocytes are responsible for the secretion of extra-cellular matrix during development, in particular around the ventral nervous system and Malpighian tubules (Olofsson et Page 2005; Bunt et al. 2010).

- Crystal cells

Crystal cells represent around 5 % of the total circulating cells. They were first identified because of the crystalline inclusions they have in their cytoplasm (M. T. M. Rizki 1957); these

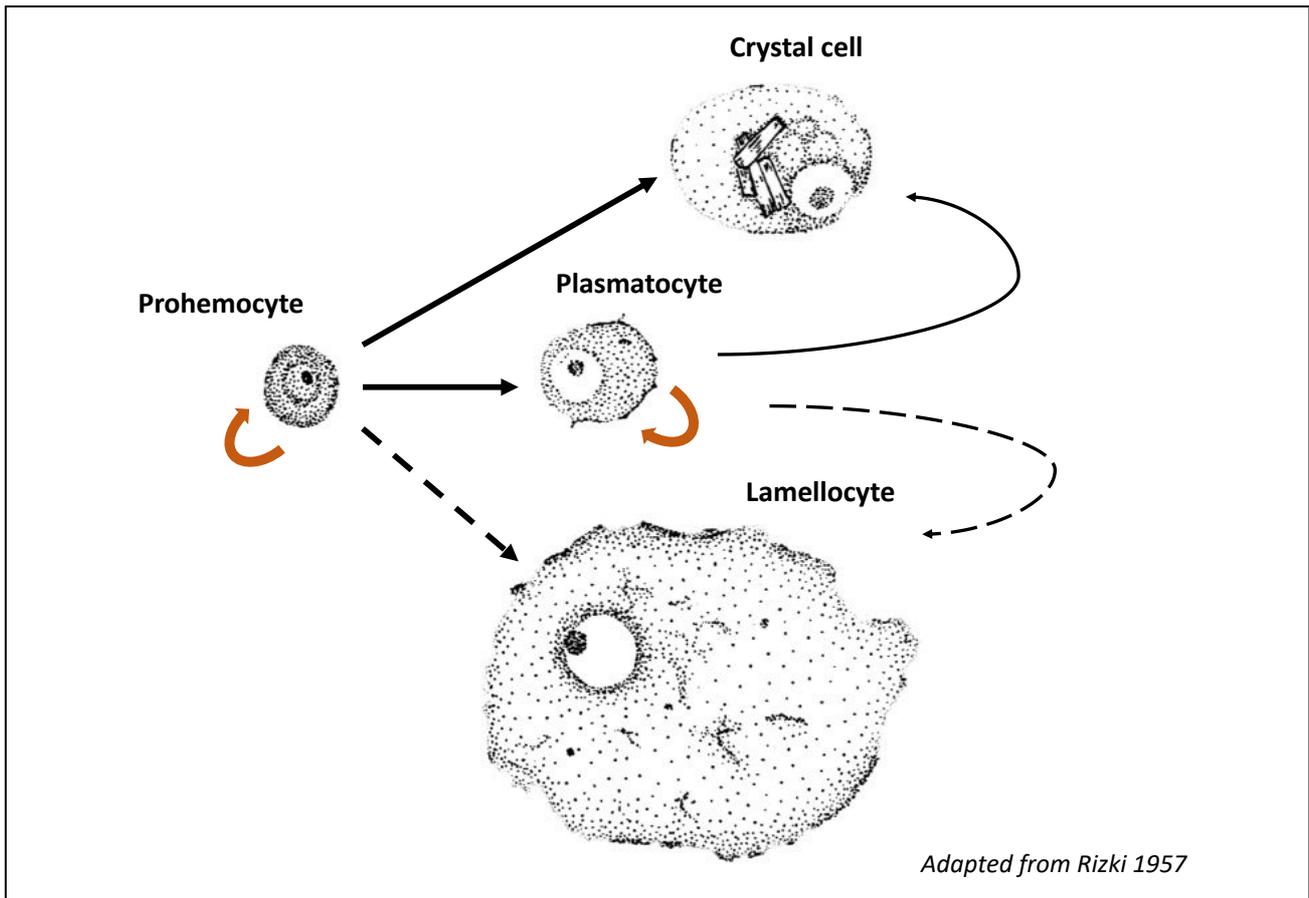


Figure 7: Drosophila blood cells

Schematic representation of blood cell types in *Drosophila*. Progenitors, or prohemocytes, give rise to plasmatocytes, which are macrophages, and to crystal cells, which have crystalline inclusions and are functional homologs of the mammalian megakaryocytes. Upon an immune challenge like wasp infestation, prohemocytes in the larva are able to differentiate into lamellocytes; these are big cells able to encapsulate the wasp egg. It also has been shown that plasmatocytes in the larval stages are able to transdifferentiate into crystal cells or upon infestation into lamellocytes. (*Adapted from Rizki 1957*)

inclusions are in fact accumulation of prophenol-oxydases (PPOs) involved in the arthropod-specific melanization reaction (Dudzic et al. 2015). Melanization reactions are responsible for wound healing by promoting coagulation; this makes crystal cells the functional homologs of mammalian megakaryocytes. These PPOs are processed into active phenoloxydases by a proteolytic cascade (Nam et al. 2012), when they are released in circulation after crystal cell rupture (Bidla, Dushay, et Theopold 2007). This activation has also been shown to contribute to survival after bacterial infection (Binggeli et al. 2014). I will describe later, in more detail, how crystal cells are formed, in particular during the larval stage.

- Lamellocytes

Lamellocytes are the third and last blood cell type in *Drosophila*. They are large cells produced specifically when parasitoid wasps lay eggs inside the larval cuticle (M. T. M. Rizki 1957; T. M. Rizki et Rizki 1992; Crozatier et al. 2004). Lamellocytes can be formed in circulation by the transdifferentiation of plasmatocytes (Avet-Rochex et al. 2010; Stofanko, Kwon, et Badenhorst 2010; Anderl et al. 2016), and also directly from progenitors in a specialized organ called the lymph gland (Oyallon et al. 2016). Their function is to encapsulate objects too big to be phagocytized. It has been shown that in a wasp infestation condition, lamellocytes arise from the pool of intermediate progenitors in the lymph gland, and that this process occurs at the expense of crystal cell formation, suggesting that the choice between the two cell types is mutually exclusive (Krzemien et al. 2010). Although lamellocytes are produced in response to an immune stress, they can also be generated both in the lymph gland and in circulation, in some genetic conditions (Avet-Rochex et al. 2010).

b) *Drosophila* blood cell formation

As for mammals, blood cells in *Drosophila* are formed during successive waves of hematopoiesis: an embryonic wave that gives rise to all circulating hemocytes in the larva, and a larval wave that generates the pool of hemocytes required during metamorphosis and later on, during adult life.

c) Embryonic wave of hematopoiesis

Blood cells in the embryo first originate from the procephalic mesoderm as bipotent progenitors (they can differentiate into both plasmatocytes and crystal cells; Tepass et al. 1994; Holz 2003). These progenitors called prohemocytes, emerge, and differentiate into the two embryonic blood cell types. Plasmatocytes then migrate throughout the embryo (Tepass et al. 1994; Wood, Faria, et Jacinto 2006), while crystal cell lineages stay in a cluster next to the proventriculus (Lebestky 2000; Bataillé et al. 2005). At the end of embryogenesis, about 700 plasmatocytes and 30 crystal cells are generated. It is important to note that since lamellocytes are produced after an immune stress like parasitism, they only appear at the larval stage and are completely absent from the embryo (Figure 8A).

Hemocytes formed after this first hematopoietic wave during embryogenesis will persist in all stages of life. Indeed, during the larval stages, plasmatocytes will aggregate in stereotyped clusters along the cuticle that will become new hematopoietic sites called hematopoietic pockets (HPs; Figure 8B). These HPs provide a microenvironment in which plasmatocytes proliferate (K. Makhijani et al. 2011; Leitão et Sucena 2015) and give rise to the pool of larval circulating hemocytes. Interestingly, these clusters of self-renewing plasmatocytes have been shown to share functional similarities with resident macrophages in mammals (reviewed in Gold et Brückner 2015). Plasmatocytes are attracted to HPs, because of chemotaxis signals produced by peripheral neurons (Kalpana Makhijani et al. 2017), and when stimulated by appropriate signals, they can differentiate into either crystal cells (Leitão et Sucena 2015) or lamellocytes (Honti et al. 2009). It also has been shown recently that plasmatocytes located near pericardial cells of the dorsal vessel, are also able to transdifferentiate into crystal cells, seemingly after Notch induction (Cevik et al. 2019). All the cells produced during this wave subsequently persist until adulthood.

d) Larval wave of hematopoiesis

The second wave of hematopoiesis, which takes place during the larval stage in a specialized organ called the lymph gland (Figure 8C; reviewed in Banerjee et al. 2019), has its

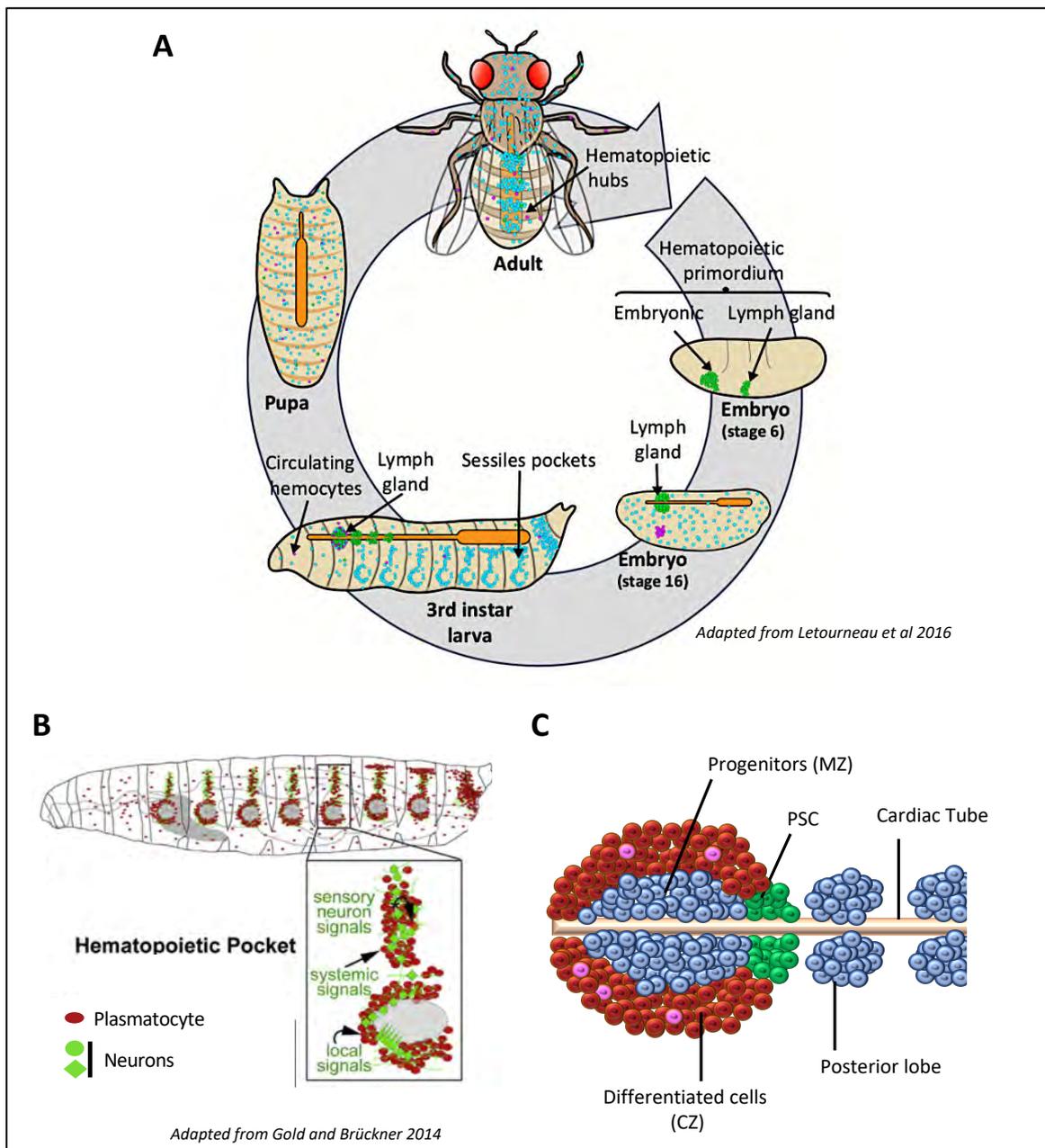


Figure 8: Drosophila life cycle and hematopoiesis

A. During embryonic development, cells of the procephalic mesoderm and cardiogenic mesoderm (in green) develop to give rise to both circulating hemocytes (blue and purple) and to the lymph gland (green), apposed on the cardiac tube (orange). The embryo develops into a larva, then a pupa and finally an adult, where hematopoiesis occurs within Hematopoietic Hubs (HB). **B.** Representation of larval hematopoietic pockets during the third larval stage. In this microenvironment, plasmatocytes (brown) are in contact with neurons (green) that emit signals to promote plasmatocyte homing and proliferation. Within this microenvironment, plasmatocytes can give rise to larval crystal cells by transdifferentiating. **C.** Schematic representation of the lymph gland during the third larval stage. In the medullary zone of the anterior pair of lobes (MZ, blue), progenitors are mostly quiescent with a low rate of proliferation. They give rise to differentiated cells (plasmatocytes, red, and crystal cells, purple) located in the cortical zone (CZ). During wasp infestation, progenitors in the MZ are instructed by the Posterior Signaling Center (PSC) to become lamellocytes. The posterior lobes (also blue) along the cardiac tube will in the adult stage become parts of the HB. (Adapted from Letourneau et al 2016; Gold and Brückner 2014).

origins in the embryo. Indeed, lymph gland progenitors emerge in the dorsal mesoderm and then migrate along the dorsal vessel. It has been shown that these precursors have a common origin with endothelial cells, both arising from hemangioblastic cells (Mandal, Banerjee, et Hartenstein 2004); this is a common feature with mammals, where hemogenic endothelium generates all blood precursors during embryonic development.

The lymph gland is a regionalized organ, with two primary lobes apposed on the cardiac tube and multiple pairs of secondary lobes along the dorsal vessel (reviewed in Letourneau et al. 2016). Primary lobes are themselves regionalized in two regions: one medullary zone in which progenitors proliferate during the first larval stages and where they remain in a mostly quiescent state in later stages, and one cortical zone where differentiated cells (plasmacytes and crystal cells) accumulate. One should note that a third zone is visible, the intermediary zone, where differentiating progenitors are found (Oyallon et al. 2016). Another compartment of interest, the Posterior Signaling Center, defines a microenvironment responsible for massive lamellocyte production after an immune stress (Benmimoun et al. 2015; Oyallon et al. 2016). In this case, the lymph gland is prematurely disrupted and releases all its content into circulation, in order to encapsulate the wasp egg and prevent its development, which would otherwise be lethal for the fly.

In the absence of parasitism, at the end of the L3 stage, the lymph gland contains only differentiated cells, which are released in the hemolymph at the onset of pupariation.

e) Hematopoiesis in the adult fly

Along with cells generated during the first hematopoietic wave, cells produced by the lymph gland will persist during the adult stage. In addition, it has been shown that there is a slight hematopoietic activity during adult life, in clusters located along the abdominal part (Saikat Ghosh et al. 2015).

Blood cells from lymph gland secondary lobes migrate in these clusters called Hematopoietic Hubs (HBs) to become progenitors of the new hematopoietic wave. Progenitors in HBs display proliferation abilities and give rise to both plasmacytes and

crystal cell lineages (S Ghosh, Mandal, et Mandal 2018), which actively participate in protecting against infection.

3. Molecular actors during *Drosophila* hematopoiesis

It has been shown in the last fifteen years that there is a strong conservation from *Drosophila* to mammals of the molecular actors regulating hematopoiesis. Indeed, key hematopoietic regulators belonging to the GATA and RUNX family of transcription factors are crucial actors during *Drosophila* hematopoiesis, as well as the Notch signaling pathway. I will describe below how they interact and participate in the establishment of blood cell lineages in *Drosophila*.

a) GATA transcription factors

In *Drosophila* there are five GATA factors: Serpent (Srp), Pannier (Pnr), GATAe, GATAd and Grain. They are major regulators of development, but only Srp and Pnr have been identified so far as playing a role during hematopoiesis (Rehorn et al. 1996; Lucas Waltzer et al. 2010; Minakhina, Tan, et Steward 2011).

Srp is absolutely required for prohemocyte emergence, as Srp mutant embryos are completely deprived of blood cells (Rehorn et al. 1996). At the cellularization stage, *srp* is expressed in the procephalic mesoderm, in the prohemocyte cluster (Spahn et al. 2014). Later in embryonic and larval development, *srp* expression is maintained in every blood cell lineage, which makes it a “panhemocyte” marker. Its interaction with its cofactor FOG U-shaped (Ush) is required for plasmatocyte differentiation, and its interaction with the RUNX factor Lz is required for crystal cell differentiation (Fossett et al. 2003; L. Waltzer 2002; 2003a; Bataillé et al. 2005).

Srp has also been shown to be involved during lymph gland hematopoiesis. It has been proposed that the interaction with its cofactor Ush is required for the normal expression of E-cadherin in the progenitor compartment to maintain their undifferentiated state.

In summary, Srp seems to share functional similarities with GATA1 and 2 in mammals, as it is required both for the emergence of prohemocytes and later on for their proper differentiation.

b) RUNX transcription factors

In *Drosophila*, the RUNX ortholog Lozenge (Lz) is a crucial transcription factor during the hematopoietic process. I will, in this part, give more details concerning its history and functions during *Drosophila* development. Lz was first identified by the group of Thomas Morgan in the early twentieth century (Morgan, Bridges, et Sturtevant 1925), and its name comes from the eye phenotype displayed by mutant flies.

Lz was initially cloned by the group of Uptal Banerjee, who showed its involvement in cell fate choice in the eye imaginal disc (Daga et al. 1996). In this study, the authors generated a series of mutant alleles, and one of them, the *lz^{R1}* allele, is the one I used in experiments I will describe in Results. Lz was linked to other processes like fertility (Anderson 1945), as well as in the regulation of phenol-oxydase activity (Peeples et al. 1969; Warner, Grell, et Jacobson 1974). Indeed, Lz was shown to be a suppressor of the *black cells* phenotype, which is caused by a mutation in the PPO1 gene (T. M. Rizki et Rizki 1981). PPOs are produced by crystal cells during embryonic and larval hematopoiesis, and it has been demonstrated that Lz is a crucial transcription factor controlling crystal cell formation (L. Waltzer 2003a).

Similarly to RUNX1, Lz can interact with several factors during the hematopoietic process. Moreover, since RUNX1 expression is initiated in Notch activated cells in the AGM, *lz* expression in larval crystal cells depends on Notch signaling, and I will describe below these interactions.

- [Interaction between Lz and Srp is crucial for crystal cell differentiation](#)

In the embryo, it has been shown that Lz physically interacts with the GATA factor Srp to promote crystal cell differentiation and transcription of target genes such as *PPO1* and 2 (L.

Waltzer 2003a; Ferjoux et al. 2007). This interaction is required to produce crystal cells, but it is also sufficient to induce *PPO* transcription ectopically in the embryo.

- CBF β factors in *Drosophila*, Brother (Bro) and Big brother (Bgb)

The CBF β factors are heterodimerization partners for RUNX transcription factors. Indeed, it has been shown that Bro and Bgb are involved during eye development in collaboration with Lz (Ling-Hui et Gergen 1999; Kaminker et al. 2001), and also in collaboration with Runt during segmentation (Kaminker et al. 2001). No function, however, of either Bro or Bgb in hematopoiesis has been reported yet.

- Notch signaling pathway

During larval hematopoiesis, crystal cell formation has been shown to be initiated and dependent on Notch signaling (Duvic et al. 2002), both in the lymph gland and in Hematopoietic Pockets (HPs). In the lymph gland, some cells in the cortical zone have been found to express the Notch ligand Serrate (Ser), and are involved in the cell-cell contact required for Notch activation; if these cells are absent of the lymph gland, there is no crystal cell differentiation (Lebestky 2003). These Ser expressing cells are called Lineage Specifying Cells (LSCs, Ferguson et Martinez-Agosto 2014). In the HP, the Notch signaling pathway is also required, as Ser expressing cells induce plasmacyte transdifferentiation into crystal cell precursors (Leitão et Sucena 2015). The Notch ligand Delta is not involved during this process, as its depletion does not impact crystal cell formation (Duvic et al. 2002; Lebestky 2003). Activation of the pathway specifies crystal cell lineage most likely *via* initiation of *lz* transcription. In turn, Lz switches on the specific crystal cell transcriptional program and maintains its expression by an autoregulatory loop (Ferjoux et al. 2007).

It has been shown that in addition to its requirement for the induction of *lz* transcription, Notch functionally interacts with Lz itself for the transcriptional activation of crystal cell specific target genes like *klumpfuss* and *pebbled* (Terriente-Felix et al. 2013; Skalska et al. 2015) thereby contributing to their differentiation. In addition to these two roles (specification and differentiation), the Notch pathway is also required for crystal cell survival. Indeed, the

pathway is maintained activated in mature crystal cells, until its *Lz*-mediated downregulation provokes crystal cell rupture (Mukherjee et al. 2011; Miller et al. 2017).

But Notch is not the only pathway involved in crystal cell differentiation; the Hippo pathway is also required for this process.

- Hippo signaling pathway

The Hippo signaling pathway is mostly known for its function during organ growth, as it is required to tightly control organ size (Moeller et al. 2017). It is also involved in cell proliferation and is sensitive to cell contact signals (Ota et Sasaki 2008).

The Hippo signaling pathway relies on a phosphorylation cascade, and most of its members are kinases. Extrinsic signals are sensed by the Tao kinase, which phosphorylates Hippo that subsequently phosphorylates Warts. Finally Warts phosphorylates Yorkie (Yki), the pathway effector, which as a consequence is sequestered in the cytoplasm (reviewed in Meng, Moroishi, et Guan 2016) where it remains inactive. When the pathway is inactive, Yki is present in the nucleus and interacts with molecular partners that are transcription factors; its principal partner is the TEAD factor Scalloped (Sd), and together they activate transcription of target genes like *DIAP-1*, which promotes cell survival (Figure 9). A recent study showed that Sd is required to regulate maintenance and proliferation of lymph gland progenitors, but it is not clear whether Yki is involved in this process or not (Ferguson et Martinez-Agosto 2017).

The same group reported that Yki is required downstream of Notch activation in the lymph gland to initiate *Lz* expression and thus allow crystal cell differentiation (Figure 10; Ferguson et Martinez-Agosto 2014). It also has been demonstrated, in cell culture with a luciferase reporter assay, that Yki is able to bind directly some enhancers in the *Lz* gene (Milton et al. 2014). This was therefore proposed to be the mechanism for the direct transcriptional activation of *Lz* by a Yki/Sd complex during crystal cell differentiation in the lymph gland.

It is noteworthy that, depending on the context, the interactions between the Notch and Hippo signaling pathways can be very different. For instance, it has been shown in the *Drosophila* wing disc that Notch signaling is able to promote Yki activity in a non cell-autonomous manner (Graves et al. 2012) to promote cell survival. However, in the wing pouch,

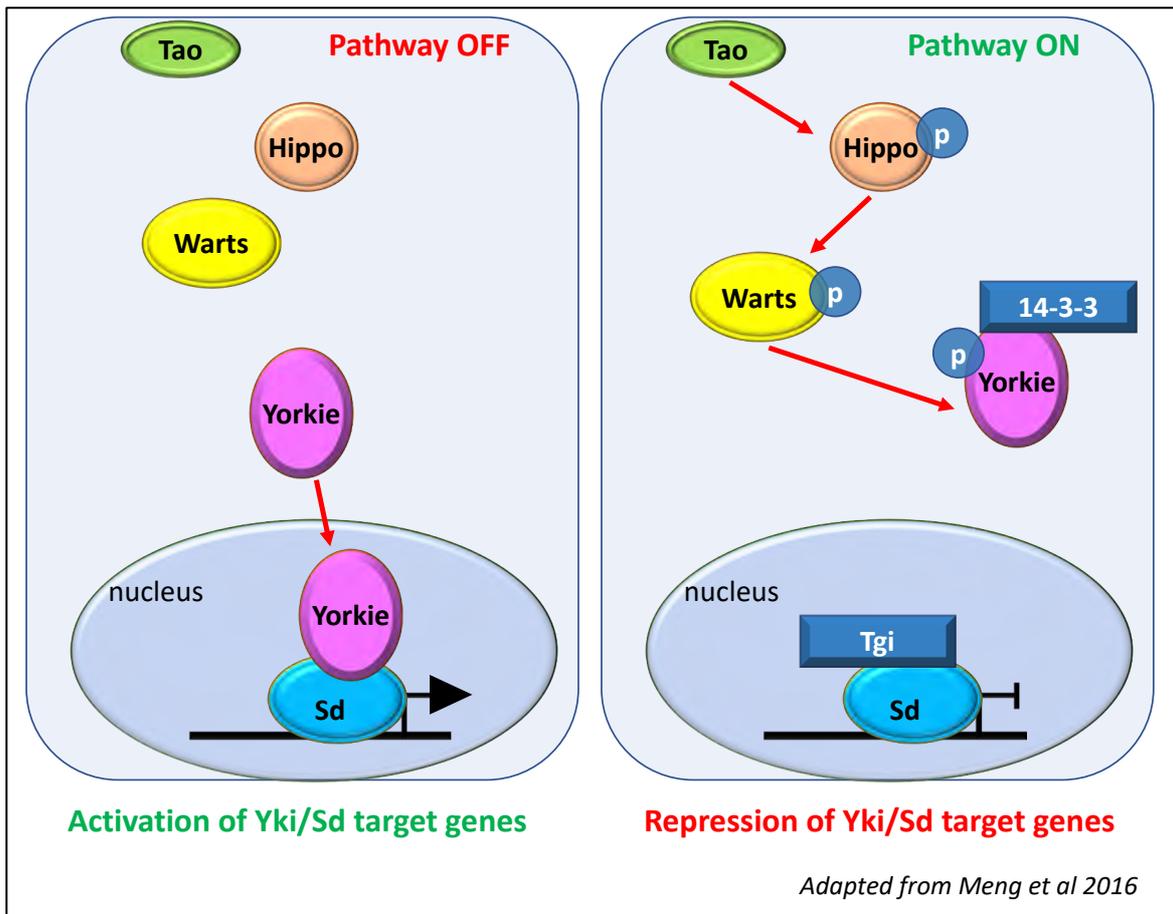


Figure 9: Overview of the Hippo signaling pathway in Drosophila

When the Hippo pathway is inactive, its effector Yorkie competes in the nucleus with Tondu domain-containing growth inhibitor (Tgi) for interaction with Scalloped (Sd) and activates transcription of target genes. The pathway is activated by the Tao kinase, which induces the phosphorylation cascade by phosphorylating Hippo. In turn Hippo phosphorylates Warts, which phosphorylates Yorkie. Yorkie is then sequestered in the cytoplasm by the interaction with 14-3-3, and Tgi interacts with Sd and represses Yorkie target genes. (Adapted from Meng et al 2016)

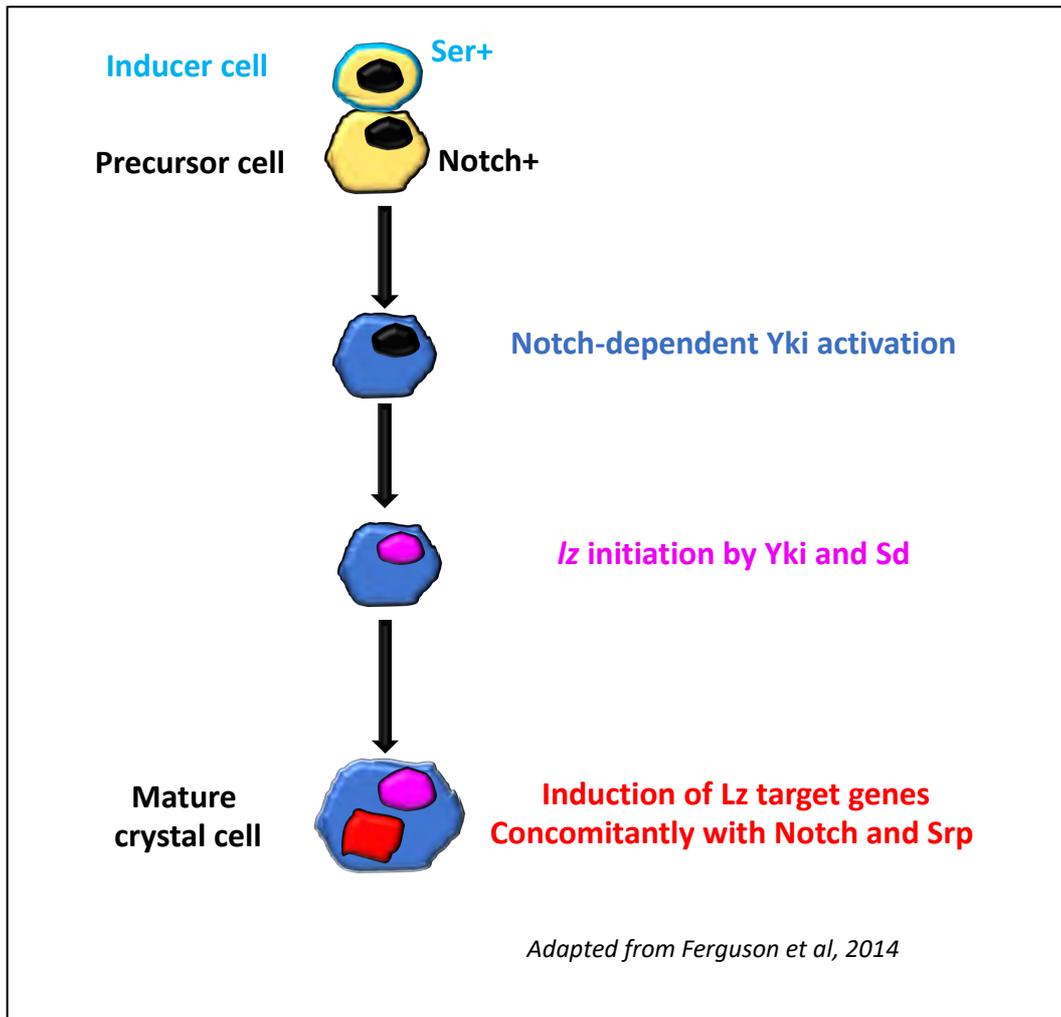


Figure 10: Differentiation of crystal cells in the lymph gland

In the model proposed for crystal cell differentiation in the lymph gland, an inducer cell expressing the Notch ligand Serrate (Ser) activates the pathway in a contacting cell, which will then become a crystal cell precursor. The activation of the pathway leads to the activation of Yorkie, which with Sd is responsible for initiating *lozenge (Lz)* expression. Finally, Lz induces the expression of its target genes in cooperation with Notch and/or Srp, thus promoting the cell's maturation. (*Adapted from Ferguson and Martinez-Agosto 2014*)

Notch activation induces the expression of Vestigial, which is a Sd partner; this consequence inhibits Yki/Sd transcriptional activity and downregulates expression of Hippo pathway target genes (Djiane et al. 2014).

4. *Drosophila* as a model for pathological hematopoiesis

Molecular actors of the hematopoietic process are highly conserved, and in particular partners of the RUNX transcription factors. This allows molecular and mechanistic studies of their functions *in vivo*, which is quite complicated in a mammalian model.

In addition to its relevance to studying the process of normal hematopoiesis and homeostasis of blood cell production, *Drosophila* is used as a model for pathological situations, as hematopoietic deregulation can be induced *in vivo*. This makes *Drosophila* an excellent tool to study the molecular mechanisms of leukemic transformation, and in the next part I will describe some of these models.

a) RUNX1-ETO as a model for human leukemic transformation

As previously mentioned, RUNX1 is a major target of single mutations or chromosomal rearrangements that lead to the development of AML. In order to gain a better understanding of the molecular mode of action by which the RUNX1-ETO fusion protein induces a leukemic transformation, two *Drosophila* models were developed.

In a first study aiming at the identification of suppressors of RUNX1-ETO activity (Osman et al. 2009), the authors overexpressed the RUNX1-ETO construct using the Gal4/UAS system in hemocytes. RUNX1-ETO overexpression impairs crystal cell differentiation in the embryo mostly due to the competition with Lz transcriptional activity on its target genes. When overexpressed during the larval stages with the *Lz-Gal4* driver, RUNX1-ETO blocks crystal cell differentiation both in circulation and in the lymph gland, without affecting Lz expression itself. This overexpression also induces a pupal lethality that was used by the authors to identify genes required downstream of RUNX1-ETO in a genetic screen. The suppressors of lethality

were tested for their ability to suppress crystal cell differentiation defects. They identified *calpainB* as required for RUNX1-ETO induced blood disorders and showed that calpain inhibition affects RUNX1-ETO protein levels in the human leukemic Kasumi-1 cell line. This study in *Drosophila*, therefore, yields new insights into the seemingly conserved molecular mechanism at work during RUNX1-ETO dependent leukemic transformation.

The second model relied on overexpression of RUNX1-ETO in all larval hemocytes (Sinenko et al. 2010). This induces a dramatic increase of hematopoietic precursors and high levels of ROS in circulation, which necessitates an interaction between RUNX1-ETO and one CBF β factor. Indeed, overexpression of a mutated version of RUNX1-ETO that is unable to bind CBF β , is not sufficient to induce the overproliferation of progenitors. This requirement for CBF β binding during RUNX1-ETO leukemic transformation, is a conserved mechanism in mammals (Roudaia et al. 2009). This increased proliferation of *Drosophila* hemocytes is reminiscent of what is observed in AML cases, because in the pathology there is proliferation of myeloid progenitors that inhibits production of normal cells. This second model allowed the identification of RUNX1-ETO activity suppressors that are involved in the antioxidant pathway in *Drosophila*, and this could be relevant to human RUNX1-ETO related AML cases.

In both studies, the authors showed that expressing the human fusion protein RUNX1-ETO in *Drosophila* was sufficient to recapitulate the major phenotypes displayed in human AML: an increased proliferation of immature progenitors on the one hand, and an inhibition of Lz/RUNX normal activity on the other hand.

b) Myeloid Leukemia Factor

The *Drosophila* Myeloid leukemia factor (Mlf) belongs to a family conserved across evolution and its mammalian homologs are involved in the development of leukemia (reviewed in Gobert, Haenlin, et Waltzer 2012). It has been shown that Mlf is required for the stability of the Lz protein (Bras et al. 2012), especially by forming a chaperone complex along with DNAJ-1 that protects Lz from degradation and ensures its accumulation in crystal cells (Dyer et al. 2017; Miller et al. 2017). By doing so, Mlf participates in the control of crystal cell differentiation, and its loss of function is accompanied by dysplastic morphology of Lz

expressing cells. In addition, Dyer et al. show that Mlf binds chromatin, and their findings suggest that MLF mediates the repression of genes associated with development and differentiation, while promoting those associated with cell proliferation (Dyer et al. 2017). Finally it also has been shown that Mlf is required for the leukemic activity of RUNX1-ETO in *Drosophila* and in the human leukemia-derived Kasumi-1 cell line (Bras et al. 2012), illustrating once again the existing molecular conservation from *Drosophila* to human.

In conclusion, over the last 15 years, *Drosophila* has proven to be a valuable model system to study hematopoiesis, whether normal or pathologic. It also appears that although the cell types and functions may be very different between mammals and insects, the crystal cell lineage represents a particularly suitable context to study the conserved basis of RUNX1-related blood cell formation.

5. Identification of *enoki mushroom* as a novel actor of *Drosophila* hematopoiesis

As described above, MOZ is a known co-activator of RUNX1 in mammalian hematopoiesis, and its deregulation leads to the development of severe blood disorders. *enoki mushroom* (*enok*) is the *Drosophila* homolog of MOZ, and they share a high homology, in particular in their N-terminal part, containing the NEMM domain, the tandem PHD fingers and the MYST domain. Given this high level of conservation, *Drosophila* seemed like an ideal model to decipher the molecular functions of this epigenetic factor.

- Identification of Enok in *Drosophila*

Enok was first identified in 2001 as a lucky side product of an Ethyl Methane Sulfonate (EMS) mutagenesis screen aiming at identifying mutations that fail to complement lethality of the *Df(2)gek^{D23}* deficiency (Scott, Lee, et Luo 2001). Two *enok* point mutation alleles were isolated: *enok¹* and *enok²*. The first allele leads to the change of a Tryptophan into a Stop codon in the MYST domain, and the second allele leads to the replacement of a Cystein in the Zinc-finger motif. Both alleles are homozygous lethal, and individuals display a developmental

delay and die at the beginning of the pupal stage. In this study, Scott *et al* showed that *enok* loss of function impairs proliferation of neuroblasts in the mushroom bodies. However, the authors hypothesized that the lethality observed in *enok* mutants is not due to the sole defect in mushroom body development. In addition, they identified a requirement for Enok in germline cells, suggesting that Enok function may affect proliferation in several tissues.

- [Enok function during establishment of the female germline](#)

In 2013, a second study led by Xin *et al* further demonstrated that *enok* is involved in female germline development (Xin et al. 2013). By inducing *enok* mutant clones specifically in germ stem cells, the authors showed that Enok function is required cell autonomously in those cells to promote their maintenance, in part by repressing *bruno* expression and thus inhibiting premature differentiation. In addition, the authors showed that Enok is required in the niche cells (called cap cells) for their maintenance *via* Notch signaling, thereby participating in the maintenance of germ stem cells in a second non-cell-autonomous manner.

- [Enok is able to acetylate histone H3, in collaboration with the ING5 complex](#)

A recent study from Jerry Workman's group showed that Enok is able *in vitro* to acetylate histone H3 on Lysine 23 (H3K23ac). In addition, they demonstrated that after induction of *enok* mutant clones in eye imaginal discs, the H3K23ac staining is almost completely lost *in vivo*; this demonstrates for the first time that Enok is responsible for histone modification. Here, the authors show that the H3K23ac mark is correlated with Enok-dependent expression of *spir* and *mael* genes, finally responsible for oocyte polarization.

Interestingly, a recent study reported that MOZ is able to acetylate H3 on Lysine 23 in Glioblastoma cell lines (Lv et al. 2017). In addition, BRPF1 is required for H3K23 acetylation *in vivo* in mouse (You et al. 2016). These findings suggest that H3K23 acetylation by the ING5 complex might be a conserved feature in gene regulation.

In a second study in 2016, Huang *et al* demonstrated that Enok catalytic activity is dependent on the entire ING5 acetylation complex (Huang et al. 2016). The complex is composed of Enok, Br140, Eaf6 and Ing5, the respective homologs of MOZ, BRPF1/2/3, EAF6

and ING5 in mammals. The authors showed in cultured S2 cells with western blot analysis that the H3K23ac levels decreased after depletion of each member of the complex. In addition, Huang *et al* showed that Enok interacts physically with Elg1 in S2 cells. Elg1 has been shown to be responsible for Proliferating Cell Nuclear Antigen (PCNA) unloading during DNA replication (Kubota et al. 2013, 1). The authors demonstrated that Enok is required to downregulate the PCNA unloading function of Elg1, in order to promote the G1/S transition, and thus prevent cell cycle arrest. Some *in vivo* experiments suggested that this interaction between Enok and Elg1 might be conserved in the female germ line, providing a new insight into Enok function in this tissue.

- [Enok and Br140 are bound on Bivalent promoters](#)

A recent study revealed that Br140, a member of the ING5 complex, binds a repertoire that largely overlaps that of the chromatin silencer Polycomb (Pc) (Kang et al. 2017). Pc is the founding member of the Polycomb group of proteins and is a part of the Polycomb Repressive Complex 1 (PRC1). PRC1 has DNA binding properties and when bound on chromatin, it can recruit Polycomb Repressor Complex 2 (PRC2), which in turn trimethylates histone H3 on lysine 27 (H3K27me3) through the activity of its catalytic subunit Enhancer of Zeste (E(z)). This histone modification is linked to gene repression and is essential for all developmental processes across evolution (reviewed in Papp 2006).

The concomitant binding of Br140 and PRC1 has been suggested as contributing to the maintenance of the bivalent state of promoters of many developmentally regulated genes in the embryo (Kang et al. 2017). Indeed, the presence of Br140 and Pc correlates with H3K4me3 and H3K27me3 chromatin marks, respectively marks of active and repressed chromatin. This chromatin landscape in mammals has been described as a specific feature of bivalent promoters (reviewed in Harikumar et Meshorer 2015). Furthermore, the authors purified the PRC1 complex and after a proteomic analysis, they observed that Enok is a partner of Pc as well as Br140, suggesting that they all form a complex to maintain chromatin in this bivalent state. In this study, the authors propose that Enok/Br140 along with PRC1 bind bivalent promoters of developmental genes. As differentiation occurs, activating or repressing factors

interact with them and allow the resolution of bivalency in favor of transcription activation or repression.

This novel and interesting model for Enok/Br140 function during development seems to be conserved across evolution, as BRPF2 and RING1B, respectively the Br140 homolog and the member of human PRC1 complex, are similarly co-bound on bivalent promoters in human embryonic stem cells (Kang et al. 2017).

In summary, the KAT Enok is involved in many developmental processes, and its main molecular partners are conserved from *Drosophila* to mammals. However, there is so far no evidence in the literature that Enok participates in *Drosophila* hematopoiesis, and our group is the first to establish a link between Enok and the regulation of Srp/Lz-dependent transcriptional activation (Gobert et al. 2010a).

- [Enok regulates the transcriptional activity of the Srp/Lz complex](#)

As I described earlier, the GATA and RUNX factors Srp and Lz act as a complex of transcription factors, both necessary and sufficient for expression of target genes such as the PPOs, and therefore for crystal cell differentiation in the embryo (L. Waltzer 2003b; Ferjoux et al. 2007). A genome-wide screen in cell culture led to the identification of modulators of the GATA/RUNX complex transcriptional activity, including *enok*. (Gobert et al. 2010).

III. Objectives of my PhD work

The genome wide screen led by our group identified the Lysine Acetyltransferase Enok as being potentially a part of the hematopoietic process. Preliminary results showed that *in vivo*, *enok* loss of function leads to a dramatic loss of crystal cells during the larval stage, establishing its involvement during blood cell formation. My project was to understand how *enok* participated in the production of crystal cells, by studying the molecular mechanisms involved in this process.

We can recapitulate the research project in three main questions:

- *Is the KAT activity of Enok involved during the hematopoietic process in Drosophila?*
- *Which are Enok partners during crystal cell development?*
- *Does Enok regulate directly or indirectly crystal cell differentiation?*

IV. Results

A. Thesis article in preparation

A catalytic-independent function of the *Drosophila* MOZ homolog Enok is required to control Notch-induced expression of the RUNX factor Lozenge.

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1. Abstract

The human KAT6 lysine acetyltransferase MOZ has been shown to be an essential player in the field of normal and malignant hematopoiesis. It belongs to a highly conserved family of epigenetic factors and remodels chromatin by acetylating histone tails in association with its partners of the ING5 complex. Here, we report that its *Drosophila* counterpart Enok is required during larval hematopoiesis to control the Notch-dependent process of plasmacyte transdifferentiation into crystal cells. In particular *enok* is essential to allow expression of the RUNX factor Lozenge (Lz) that controls the crystal cell specific transcriptional program. We demonstrate that this function involves neither the Eaf6 and Ing5 subunits of the *Drosophila* ING5 complex, nor its own acetyltransferase activity. We provide evidence that Enok binds a new *lz* enhancer specifically required for its expression in Notch-activated crystal cell precursors. We propose that DNA-binding properties of KAT6 proteins might represent an alternative mechanism to support catalytic-independent activities in hematopoiesis.

2. Introduction

Hematopoiesis, the highly dynamic process of all blood cell production, is tightly regulated by key transcription and epigenetic factors (Menegatti *et al*, 2019; Fujiwara, 2017; Hu & Shilatifard, 2016). Many of these regulators have been identified through the pathologies induced by their deregulation/mutation. In particular, genetic aberrations such as chromosomal rearrangements have been instrumental, since they allowed immediate identification of the targeted loci. As an example, characterization of the leukemogenic translocation t(8;21) led to the discovery of the transcription factor RUNX1 that was further shown to be essential for multiple steps of mammalian hematopoiesis (Lam & Zhang, 2012). Similarly, the gene encoding Monocytic Leukemia Zinc finger protein (MOZ, also known as KAT6A) was originally identified as the target of myeloid leukemia associated rearrangements, which fuse it to Lysine Acetyltransferases (KAT) such as CREB-binding protein (CBP) or p300, or to the coactivator TIF2 (reviewed in (Katsumoto *et al*, 2008)).

MOZ/KAT6A belongs to a family of KATs conserved from yeast to human, the MYST family (named after its founding members MOZ/Ybf2/Sas2/Tip60), and is mainly known for its ability to acetylate histone H3 on lysine-9, lysine-14 and lysine-23 residues (Voss *et al*, 2009; Qiu *et al*, 2012; Dreveny *et al*, 2014; Lv *et al*, 2017). It is therefore proposed to control transcriptional events by participating in chromatin remodeling *via* the modification of epigenetic marks. MOZ exerts its acetylation activity in a tetrameric ING5 complex also containing the bromodomain PHD finger protein 1 (BRPF1), the human Esa1-associated factor 6 homolog (hEAF6), and the inhibitor of growth 5 (ING5) subunits (Doyon *et al*, 2006; Ullah *et al*, 2008). The analysis of a MOZ mutant murine model specifically deprived of its catalytic activity (Perez-Campo *et al*, 2009) reveals a drastic reduction in hematopoietic stem cells (HSCs) and committed precursor populations, directly resulting from their reduced proliferation capacities. These results highlight the essential role of MOZ-driven acetylation in HSCs. However, the phenotypic differences existing between this catalytically inactive MOZ mutant and a loss of the entire MOZ protein (Katsumoto *et al*, 2006) suggest that MOZ may also ensure non-acetyltransferase functions *in vivo*, consistent with previous observations that MOZ interaction with RUNX1 promotes transcription independently of its catalytic domain in murine cultured cells (Kitabayashi *et al*, 2001).

Enoki mushroom (Enok), *Drosophila melanogaster* MOZ homolog, was first identified for its role in neuroblast proliferation (Scott *et al*, 2001). Its loss-of-function results in atrophied mushroom bodies in the fly brain, earning the gene its name, as well as a developmental delay and lethality at the early pupal stage. *enok* was later shown to be required for visual system wiring (Berger *et al*, 2008) and for germline stem cell maintenance, a process during which it ensures both cell autonomous and non-autonomous functions (Xin *et al*, 2013). The first insights into the molecular mechanisms underlying its different roles came from a study that demonstrated Enok's ability to acetylate histone H3 on its lysine-23 (H3K23) (Huang *et al*, 2014); in addition, it showed that *enok* is required for the expression of oocyte-polarizing genes and proposed that H3K23 acetylation of its targets accounts for the contribution of *enok* to transcriptional activation. Finally, a second study by the same group, demonstrated that *Drosophila* homologs of the mammalian ING5 complex Br140, Eaf6 and Ing5 interact physically and functionally with Enok for H3K23 acetylation, and identified a new role for Enok in cell cycle regulation (Huang *et al*, 2016). Of note, no function for Enok in hematopoiesis has ever been reported.

Drosophila is a well-established model to study both normal and pathologic hematopoiesis, since its much simpler hematopoietic system displays a high degree of conservation, whether at the functional or at the molecular level (for an exhaustive review, see (Banerjee *et al*, 2019)). Reminiscent of mammalian hematopoiesis, the fly blood cells (called hemocytes) are formed in two temporally distinct waves. The embryonic wave gives rise to two differentiated cell types: plasmatocytes that mainly ensure phagocytic functions, and crystal cells that are responsible for melanization reactions and therefore contribute to wound healing and immune response. During larval life, a second wave of hematopoiesis in the lymph gland yields a reservoir of plasmatocytes and crystal cells that remain separated from the circulation until they are released at the onset of metamorphosis (under normal conditions) or mobilized in response to a parasitic infestation (Letourneau *et al*, 2016). Cells formed during the embryonic wave persist in circulation throughout larval stages until adulthood (Holz *et al*, 2003). During larval life, a massive expansion of the plasmatocyte population occurs in segmentally dispatched clusters beneath the epidermis, called hematopoietic pockets (Makhijani & Brückner, 2012). In addition, recent live-imaging of sessile hemocytes within these hematopoietic pockets shows that upon Notch signaling

pathway activation, plasmacytes transdifferentiate into crystal cells (Leitão & Sucena, 2015) as they start to express the crystal cell lineage-specific RUNX transcription factor Lozenge (Lz), thus ensuring *de novo* larval crystal cell formation.

Here we provide the first evidence that like its mammalian counterpart, Enok participates in hematopoiesis, since we show that the transdifferentiation of plasmacytes into larval circulating crystal cells is hindered in *enok* mutants. In particular, we demonstrate that the expression of Lz is dependent on a cell-autonomous function of Enok in Notch-activated crystal cell precursors. Unexpectedly, we establish that Enok is required independently of its KAT activity and of the ING5 acetylation complex and provide the prime example of a catalytic-independent function for a MOZ protein *in vivo*. Finally, we find that Enok binds a previously unidentified hematopoietic enhancer that controls expression of *lz* in crystal cell precursors, and we propose that the Enok non-catalytic mode of action relies on its binding to DNA.

3. Results

***enok* is essential for larval crystal cell formation.**

In order to assess a possible function of *enok* during *Drosophila* hematopoiesis, we examined blood cell formation during embryonic and larval life in *enok* loss-of-function mutants. The three different alleles tested (*enok*¹, *enok*² and *enok*^{Q253}), placed either in hemizygote conditions (combined to the *Df(2R)BSC155* deficiency that uncovers *enok* locus) or in allelic combination (*enok*¹/*enok*^{Q253}), exhibited a dramatic loss of circulating crystal cells at the larval stage. Functional crystal cells in hematopoietic pockets of third instar larvae can be easily visualized, as heat exposition triggers melanization reactions within the cells, which then appear as little black dots. We observed almost no melanized crystal cells in the four *enok* mutant contexts tested as compared to control situations (Fig. 1A), indicating a defect in lineage specification or in precursor cell differentiation. As circulating hemocytes continuously enter and exit hematopoietic pockets during larval life, we examined *enok* mutant hemolymph cell content. To visualize the crystal cell lineage, we used a Notch Response Element reporter construct (*NRE-GFP*), whose expression is detected in crystal cell precursors and persists until their terminal differentiation (Leitão & Sucena, 2015; Miller *et al*, 2017), and an antibody raised against Prophenoloxidase1 (PPO1), a crystal cell terminal differentiation marker. Our results show that Notch-activated hemocytes (*NRE-GFP*⁺ cells) have lost PPO1 expression in *enok* loss-of-function contexts (Fig. 1B), confirming the absence of mature crystal cells in the sessile/circulating compartment of *enok* loss-of-function larvae.

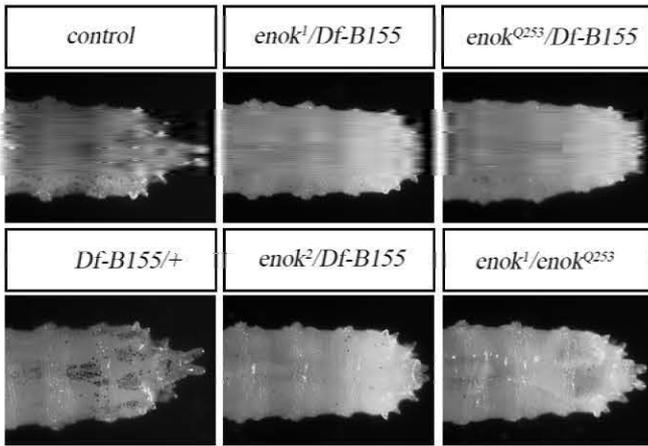
***enok* is required for transdifferentiation of plasmatocytes into larval crystal cells.**

Since the expression of PPO1 depends on the RUNX transcription factor Lz (Waltzer *et al*, 2003; Gajewski *et al*, 2007), we assessed Lz expression in circulating cells and observed that only a small fraction *NRE-GFP*⁺ cells retain the ability to express Lz in *enok* loss-of-function mutants as compared to control larvae (Fig. 1C,D). Moreover, an increased proportion of Notch-activated hemocytes displays expression of the plasmatocyte specific marker P1 (Fig. 1E), in agreement with a previous study reporting that together with Notch, Lz controls the expression of *klumpfuss*, a repressor of plasmatocyte identity (Terriente-Felix *et al*, 2013).

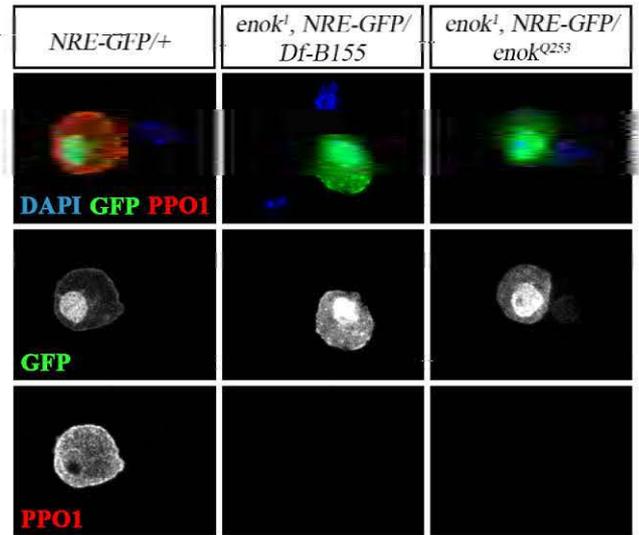
Figure 1. *enok* is required for plasmatocyte transdifferentiation into larval crystal cells. (A) Melanization assay on larvae from different *enok* mutant contexts. Posterior side of larvae is to the right. Melanized crystal cells appear as black dots. (B-C) Immunofluorescent staining on circulating cells from larvae of the indicated genotypes (*Df-B155*: abbreviation for *Df(2R)BSC155*); cells were stained with DAPI and either a-PPO1 (B) or a-Lz (C) antibody. (D) Quantification of the proportions of NRE-GFP⁺ cells expressing Lz as revealed by an immunofluorescent staining. Each dot represents the proportion observed in an individual larva. Complete genotypes: *NRE-GFP/+* (control) and *enok¹,NRE-GFP/Df(2R)BSC155* (*enok¹/Df-B155*). (E) Quantification of the proportions of NRE-GFP⁺ cells expressing the plasmatocyte specific marker P1 as revealed by an immunofluorescent staining. (B-E) Hemocytes were collected on larvae carrying a *NRE-GFP* transgene. (D,E) Statistics: **** indicate a p-value ≤ 0.0001.

Figure 1

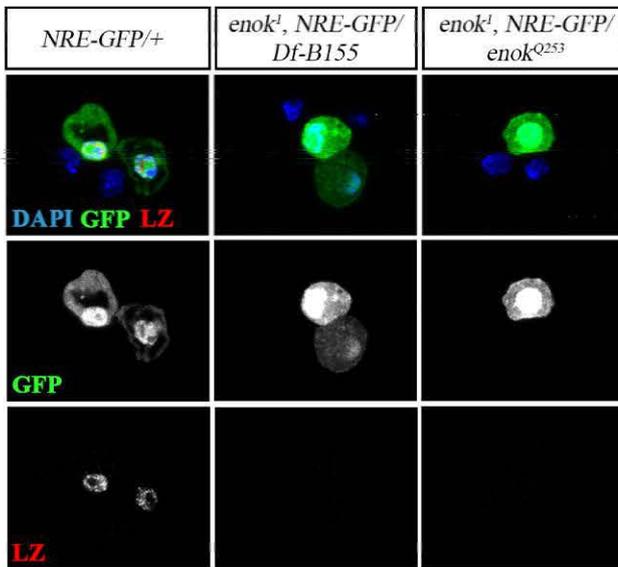
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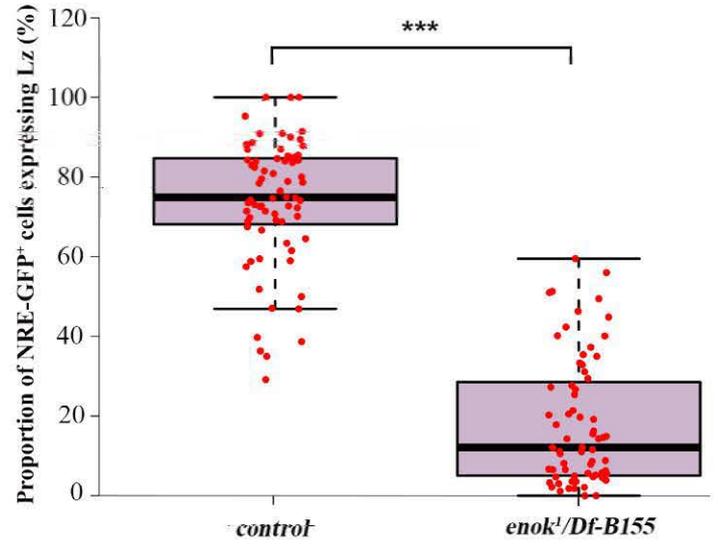
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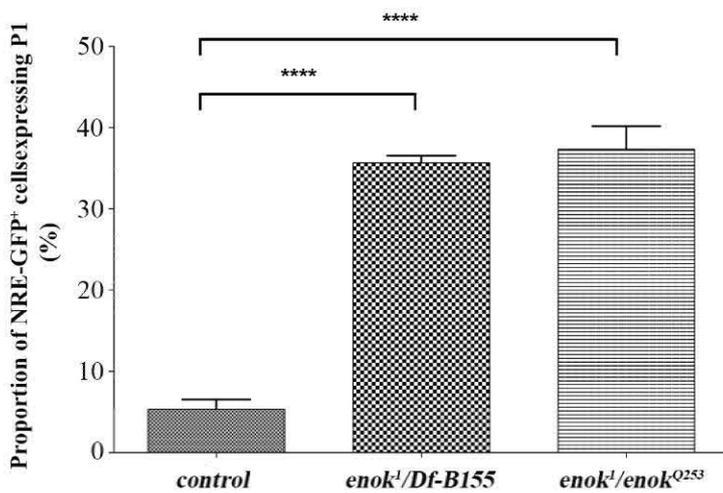
C



D



E



While we detected a minimal increase in the number of NRE-GFP⁺ cells in *enok* mutants as compared to control larvae, it was not the case with two other Notch pathway reporter constructs, rendering unlikely that the loss of Lz expression results from a generic problem in Notch signaling interpretation (Supplemental Fig. S1A-C). Altogether our results show that *enok* is required downstream of Notch signaling for Lz expression during the transdifferentiation of plasmatocytes into crystal cells.

***enok* regulates Lz expression in crystal cell precursors in a cell-autonomous fashion.**

We next sought to determine in which cells *enok* is required to promote crystal cell production. Directing expression of a *UAS-3HA-enok* transgene in *enok* mutant larvae, either ubiquitously with the *tubulin-Gal4* driver or with the *Notch^{GMR30A01}-Gal4* driver, which allows specific expression in Notch-activated crystal cell precursors (Supplemental Fig. S2A,B), restores normal crystal cell maturation in hematopoietic pockets (Fig 2A), further demonstrating that the loss of crystal cell lineage is due to the loss of *enok* function. In addition, our result indicates that *enok* ensures a cell-autonomous function in the Notch-activated precursors. Furthermore, thorough quantification of the proportion of Lz-expressing cells in circulation confirms that re-expressing *enok* specifically in crystal cell precursors of *enok* mutant larvae is sufficient to restore Lz expression completely (Fig. 2B). We thus conclude that *enok* controls the transdifferentiation of plasmatocytes into crystal cells through a cell-autonomous regulation of Lz expression in Notch-activated precursors.

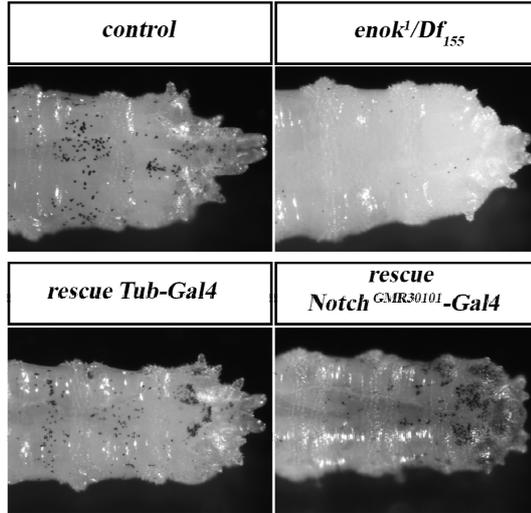
The catalytic activity of Enok and the ING5 complex are dispensable for crystal cell formation.

Since Enok is known to cooperate with its partners in the *Drosophila* ING5 complex to acetylate H3K23 (Huang *et al*, 2014, 2016), we surmised that Enok and the whole ING5 complex might control *lz* expression in Notch-activated hemocytes *via* histone acetylation. While a *Br140^{S781}* loss-of-function allele already existed, no mutation for *Eaf6* or *Ing5* was available. We thus used the CRISPR/Cas9 genome-editing system to excise most of the coding region of these two genes and recovered the *Eaf6^{M26}* and *Ing5^{ex1}* alleles (methods). Likewise, we introduced in the coding sequence of *enok* a single amino acid substitution that specifically

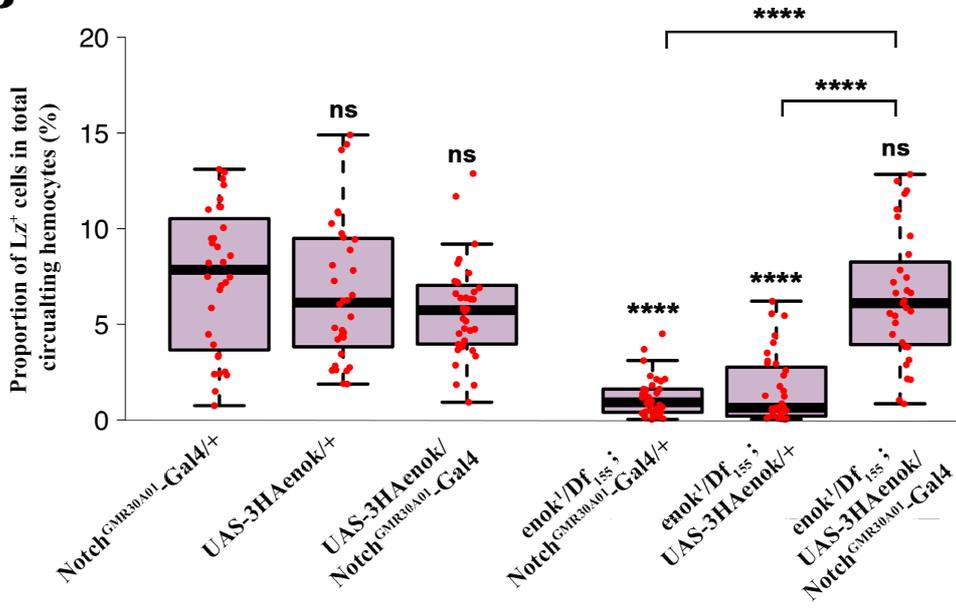
Figure 2. *Notch*^{GMR30A01}-*Gal4*-driven expression *UAS-3HA-enok* transgene in *enok* mutants restores normal crystal cell differentiation. (A) Melanization assay on larvae of the indicated genotypes. Complete genotypes: *w*¹¹¹⁸ (control), *enok*¹/*Df(2R)BSC155* (*enok*¹/*Df*-B155), *enok*¹/*Df(2R)BSC155*; *tubulin-Gal4/UAS-3HA-enok* (rescue tub-Gal4) and *enok*¹/*Df(2R)BSC155*; *Notch*^{GMR30A01}-*Gal4/UAS-3HA-enok* (rescue *Notch*^{GMR30A01}-Gal4). (B) Quantification of the proportions of total hemocytes expressing Lz as revealed by immunofluorescent staining. Each dot represents the proportion of Lz⁺ cells in an individual larva. Statistics: *Notch*^{GMR30A01}-*Gal4/+* genotype was used as a reference sample; **** indicate a p-value ≤ 0.0001, ns: not significantly different from reference sample.

Figure 2

A



B



abrogates its lysine acetyltransferase function (*enok*^{KAT} allele, see methods). As expected, the level of H3K23 acetylation in circulating cells is strongly reduced in *enok*¹ and *Br140*^{S781} hemizygote contexts and more mildly in *Eaf6*^{M26} hemizygote or *Ing5*^{ex1} homozygote mutants (Supplemental Fig. S3); of note, the *enok*^{KAT} mutation reduces the level of H3K23 acetylation to that observed with the *enok* null mutant context (*enok*¹/*Df(2R)BSC155*) (SuppFig. 3), suggesting that the *enok*^{KAT} allele is a genetically null allele for the acetyltransferase function. We then observed in melanization assays that, while *Br140* loss-of-function results in the same hematopoietic phenotype as *enok* loss-of-function, gene excision of *Eaf6* or *Ing5* did not affect larval crystal cell formation (Fig. 3A). More strikingly, *enok*^{KAT} mutant larvae exhibited seemingly normal crystal cell differentiation in hematopoietic pockets (Fig. 3A). Additional quantification of the proportion of NRE-GFP⁺ cells expressing Lz confirms that both *Br140*^{S781} and *enok*¹ mutations prevent Notch-activated precursors from differentiating further, while *enok*^{KAT} mutation has no effect on this lineage (Fig. 3B). Thus, our results demonstrate that Enok and Br140 regulate Lz through to a non-canonic mode of action that is independent of the KAT activity of Enok, and of the ING5 complex.

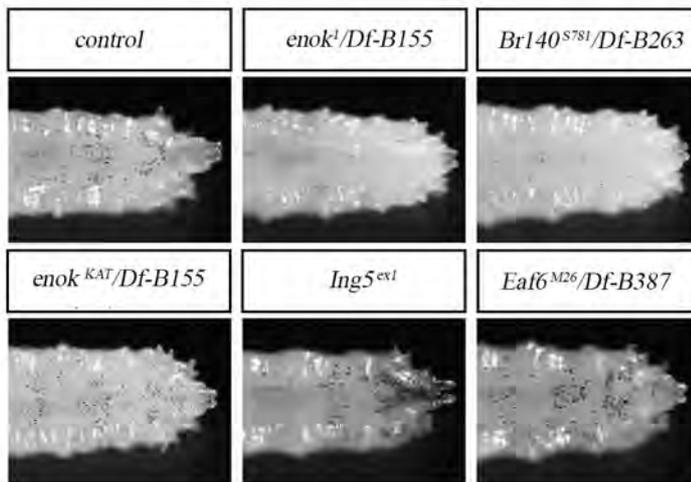
Lz expression precedes that of Yorkie during plasmatocyte to crystal cell transition.

During *de novo* hematopoiesis in the lymph gland, the onset of *lozenge* expression was reported to be under the control of the Hippo signaling pathway effector, Yorkie (Yki), following Notch signaling activation (Milton *et al*, 2014; Ferguson & Martinez-Agosto, 2014b, 2014a). We postulated that the same mechanism could be at work during larval plasmatocyte transdifferentiation in the sessile/circulating compartment. We thus performed double immunostainings to reveal both Yki and Lz expression in circulating cells of *NRE-GFP* third instar larvae. This should reveal three cell populations: (i) those expressing only NRE-GFP (precursors before they initiate differentiation), (ii) cells expressing both NRE-GFP and Yki (before they initiate Lz expression) and (iii) cells expressing the three markers NREGFP, Yki and Lz (in which Lz expression has been initiated by Yki). Our results show that most Notch-activated circulating cells indeed express Yki in a control genetic context and that this expression is lost in *enok* mutant conditions (Fig. 4A,B), indicating that *enok* is also required for Yki expression. Surprisingly however, in control larvae the vast majority of cells expressing

Figure 3. *enok* and *Br140* are required independently of the ING5 complex for crystal cell differentiation. (A) Melanization assay on larvae of the indicated genotypes (*Df-B263*: abbreviation for *Df(2R)BSC263*, *Df-B387*: abbreviation for *Df(3L)BSC387*). (B) Quantification of the proportions of NRE-GFP⁺ cells expressing Lz as revealed by immunofluorescent staining. Each dot represents the proportion of Lz⁺ cells in an individual larva. Complete genotypes: *NRE-GFP/+* (control), *enok*¹, *NRE-GFP/Df(2R)BSC155* (*enok*¹/*Df*-B155), *Br140*^{S781}, *NRE-GFP/Df(2R)BSC263* (*Br140*^{S781}/*Df*-B263) and *enok*^{KAT}, *NRE-GFP/Df(2R)BSC155* (*enok*^{KAT}/*Df*-B155). Statistics: control genotype was used as a reference sample; **** indicate a p-value ≤ 0.0001, ns: not significantly different from reference sample.

Figure 3

A



B

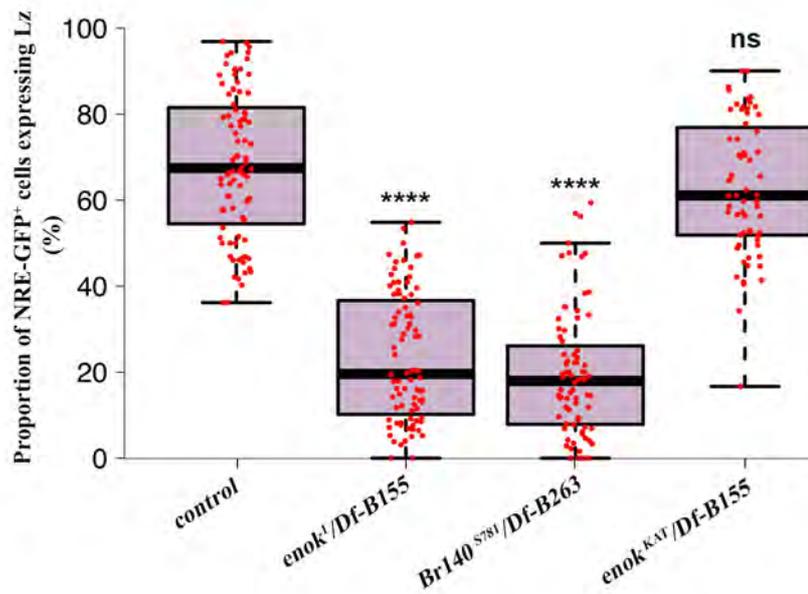
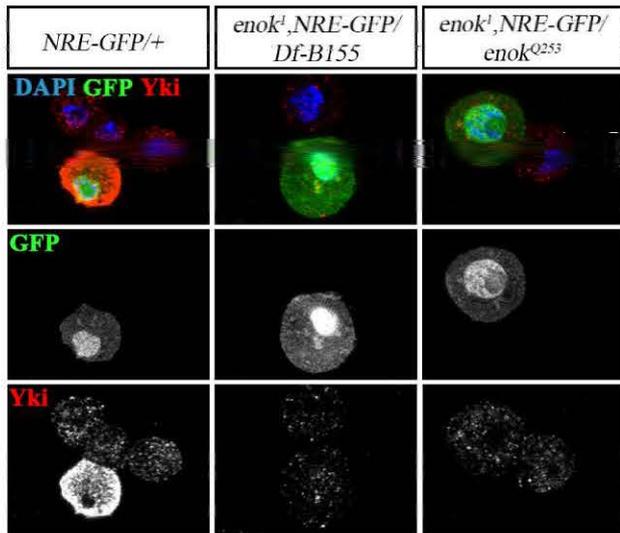


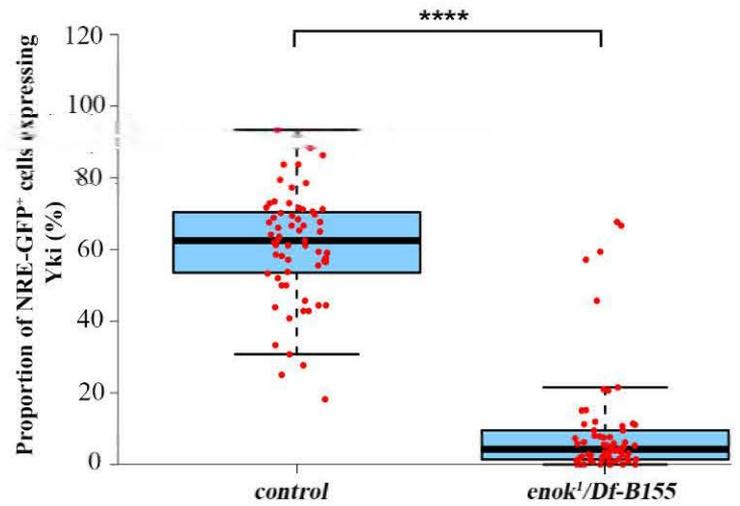
Figure 4. Lz precedes Yki expression during plasmatocyte transdifferentiation into crystal cells. (A) Immunofluorescent staining on circulating cells from larvae of the indicated genotypes; cells were stained with DAPI and α -Yki. (B) Quantification of the proportions of NRE-GFP⁺ cells expressing Yki as revealed by immunofluorescent staining. Complete genotypes: *NRE-GFP/+* (control) and *enok¹,NRE-GFP/Df(2R)BSC155* (*enok¹/Df-B155*). (C) Relative fluorescence intensity of the *NRE-GFP* transgene plotted against the relative fluorescence intensity of Yki staining in total hemocytes of control larvae; blue dots are cells negative for Lz expression and red dots are cells positive for Lz expression. The vertical dashed line represents the threshold for NRE-GFP positive cells (upper part) and the horizontal dashed line represents the threshold for Yki positive cells (right part). (D) Relative fluorescence intensity of the *NRE-GFP* transgene plotted against the relative fluorescence intensity of Lz staining in total hemocytes of control larvae; blue dots are cells negative for Yki expression and red dots are cells positive for Yki expression. The vertical dashed line represents the threshold for NRE-GFP positive cells (upper part) and the horizontal dashed line represents the threshold for Lz positive cells (right part). The dashed ellipse encloses an unexpected population of cells that express both the *NRE-GFP* reporter and Lz, but that do not express Yki. (E) Fluorescence intensity of Yki staining in NRE-GFP⁺ cells (relative to the mean Yki intensity measured in all NRE-GFP⁺ cells of control larvae) in larvae of the following genotypes: *w¹¹¹⁸/Y; NRE-GFP/+* (control) and *lz^{R1}/Y; NRE-GFP/+* (*lz^{R1}*). (F) Overexpression of *UAS-lz*, *UAS-yki* or *UAS-yki** using the *hml^D-Gal4* driver. Left panel: proportions of total circulating hemocytes expressing Lz. Right panel: proportions of total circulating hemocytes expressing Yki. Complete genotypes are as follows: *w/w; hml^D-Gal4/+* (control), *w/UAS-lz; hml^D-Gal4/+* (*UAS-lz*), *w/w; hml^D-Gal4/UAS-yki-GFP* (*UAS-yki*) and *w/w; hml^D-Gal4/+; UAS-yki^{S168A}-GFP/+* (*UAS-yki**). (B,F) Each dot represents the proportion observed in an individual larva. (C,D,E) Each dot represents the values attributed to a single cell. (B,E,F) Statistics: control genotype was used as a reference sample; **** indicate a p-value \leq 0.0001; *** indicate a p-value \leq 0.0010, ns: not significantly different from reference sample.

Figure 4

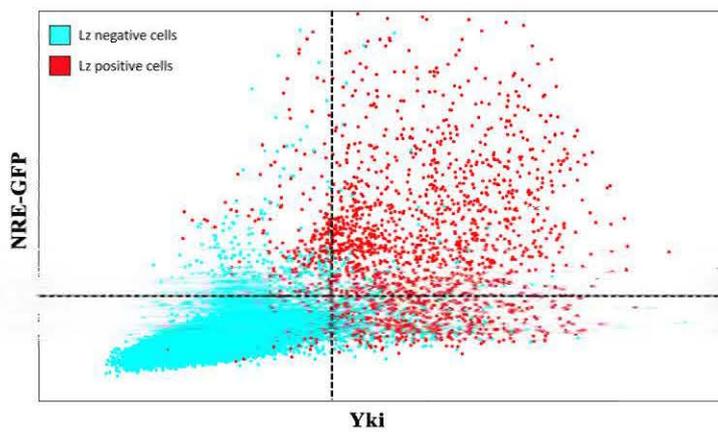
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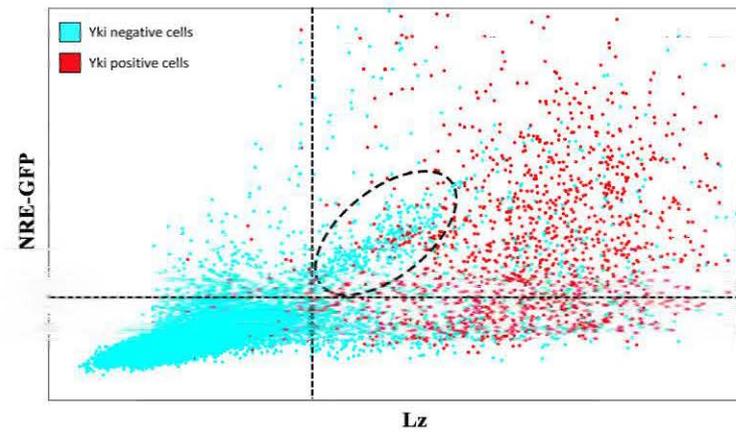
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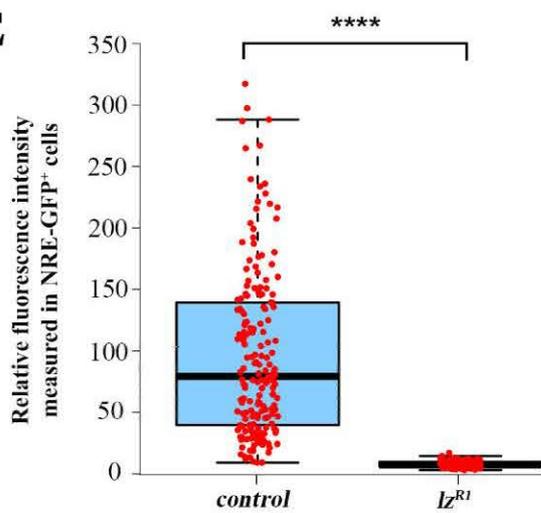
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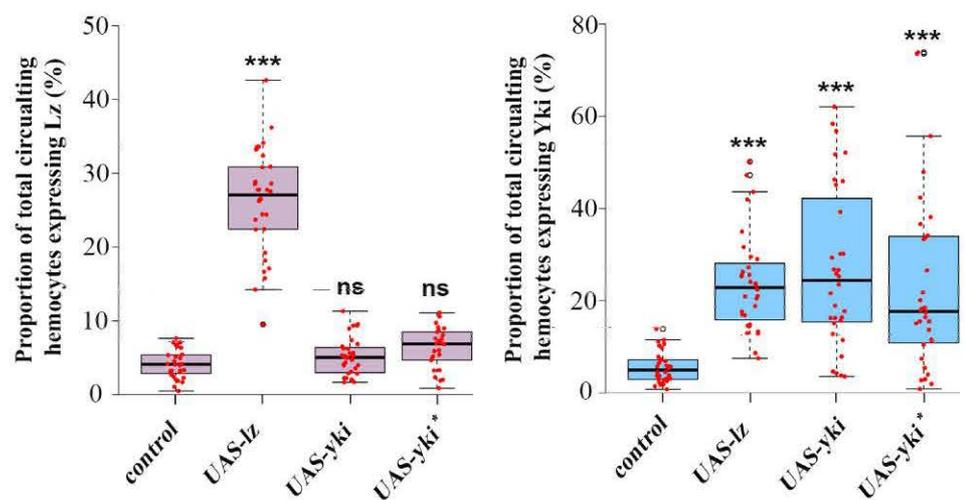
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E



F



both NRE-GFP and Yki also express Lz (Fig. 4C), whereas a subset of cells that co-express NRE-GFP and Lz appear negative for Yki staining (Fig. 4D, population in the dotted ellipse). The existence of this subpopulation does not fit the model developed for *de novo* crystal cell differentiation in the lymph gland and raises the possibility that the transcriptional cascade controlling plasmacyte transdifferentiation into crystal cells might be different, which prompted us to investigate it further. Quantification of Yki expression intensity in *Iz^{R1}* null mutant larvae shows that crystal cell precursors deprived of *Iz* do not express Yki, as compared to a control situation (Fig. 4E), indicating that *Iz* is required for Yki expression in these cells. Furthermore, ectopically expressing *Iz* in all larval circulating hemocytes with the *hemolectin-gal4* driver is sufficient to induce a significant increase of the Yki expressing cell population (Fig. 4F). In contrast, ectopic expression of Yki or activated-Yki (Yki*) does not trigger Lz expression in circulating cells (Fig. 4F). Altogether our results suggest that following Notch signaling pathway activation in plasmacytes, *Iz* expression precedes that of *yki*, and is initiated independently of the latter. We subsequently focused on the control of Lz expression by *enok*.

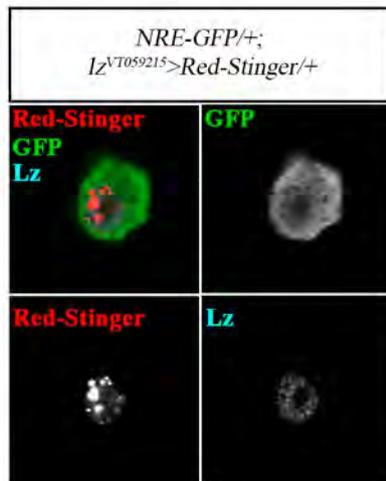
Enok directly binds an intronic region of *lozenge*, which is required for its expression in crystal cell progenitors.

As it was reported that in addition to bearing the KAT-activity the MYST domain of MOZ has the ability to bind DNA (Holbert *et al*, 2007), we hypothesized that Enok could control *Iz* expression by directly binding its regulatory regions. We selected a set of transgenic lines containing potential regulatory regions placed upstream of the Gal4 coding sequence and tested them for their ability to recapitulate *Iz* expression in the NRE-GFP expressing cells (Table 1). It appears that the regulatory region covered by the Vienna Tile VT059215, either placed upstream of the Gal4 coding sequence (*Iz^{VT059215}-Gal4*, Fig. 5A) or directly fused to the RedStinger reporter gene (*Iz^{VT059215}-RedStinger*, Fig. 5B) is able to drive transcription in a large fraction of the NRE-GFP expressing cells, which overlaps importantly but not exclusively with endogenous Lz expression (Fig. 5A,B). This potential regulatory region is embedded in the third intron of the *Iz* gene. In order to further establish the role played by this regulatory region, we decided to delete *Iz* third intron using the CRISPR/Cas genome editing technique (*Iz^{int3}*

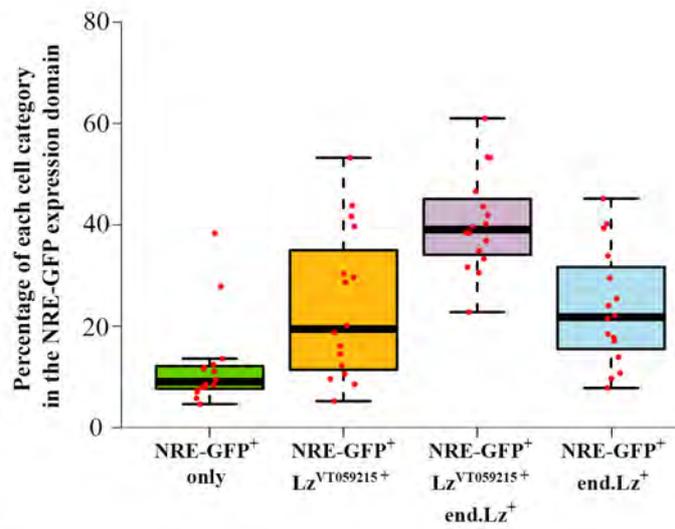
Figure 5. Enok binds a hematopoietic enhancer in *lz* third intron. (A) Immunofluorescent staining on circulating cells from *NRE-GFP/+; lz^{VT059215}-Gal4,UAS-RedStinger/+* larvae; cells were stained with DAPI and a-Lz antibody. (B) Proportions of the different categories of NRE-GFP⁺ cells quantified in *NRE-GFP/+; lz^{VT059215}-RedStinger/+* larvae. Each dot represents the proportion of the indicated cell type observed in an individual larva. (C) Immunofluorescent staining on circulating cells from larvae of the indicated genotype; cells were stained with DAPI and a-Lz antibody. (D) Quantification of the proportion of NRE-GFP⁺ cells expressing Lz in *lz^{int3}* and *control* male larvae, as revealed by immunofluorescent staining. Each dot represents the proportion of Lz⁺ cells in an individual larva. (E) Melanization assay on larvae of the indicated genotypes. (F) Quantification of the proportion of total hemocytes expressing Lz as revealed by immunofluorescent staining. Each dot represents the proportion of Lz⁺ cells in an individual larva. Statistics: *w/Y; lz^{VT059215}-Gal4/+* genotype was used as a reference sample. (G) Top: schematic representation the *lz* locus (not to scale); in purple are represented the amplicons used in Chromatin Immunoprecipitation (ChIP). Bottom: results of ChIP experiments performed on *tub-Gal4/UAS-3HA* (3HA, orange histograms) and *tub-Gal4/UAS-3HA-enok* whole embryos (3HA-Enok, blue histograms) using an a-HA antibody. (D,F,G) Statistics: *** indicate a p-value ≤ 0.0001, * indicate a p-value ≤ 0.0500, ns: not significantly different from reference sample.

Figure 5

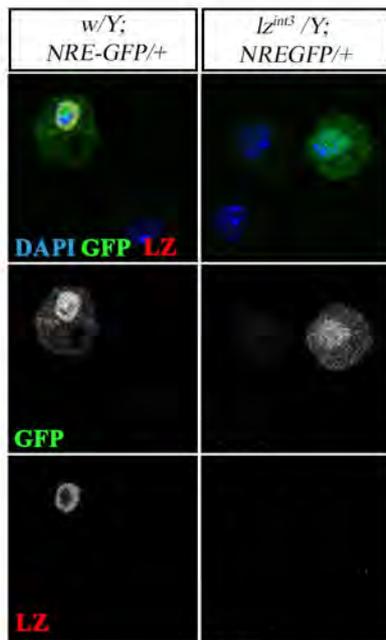
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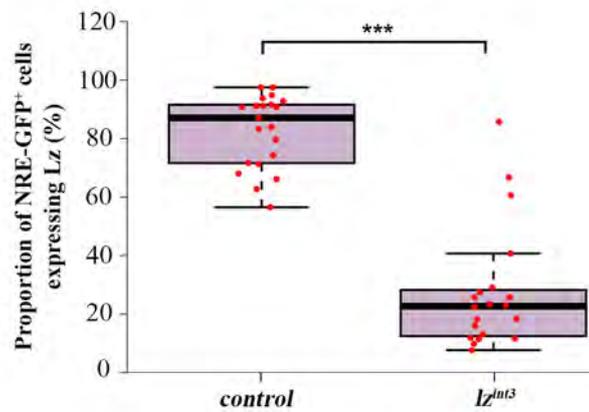
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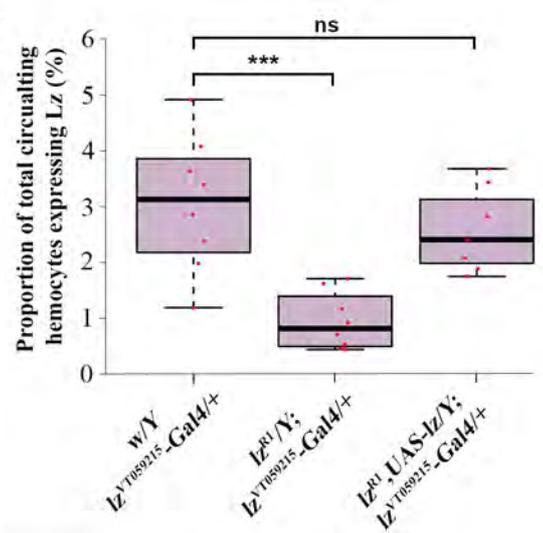
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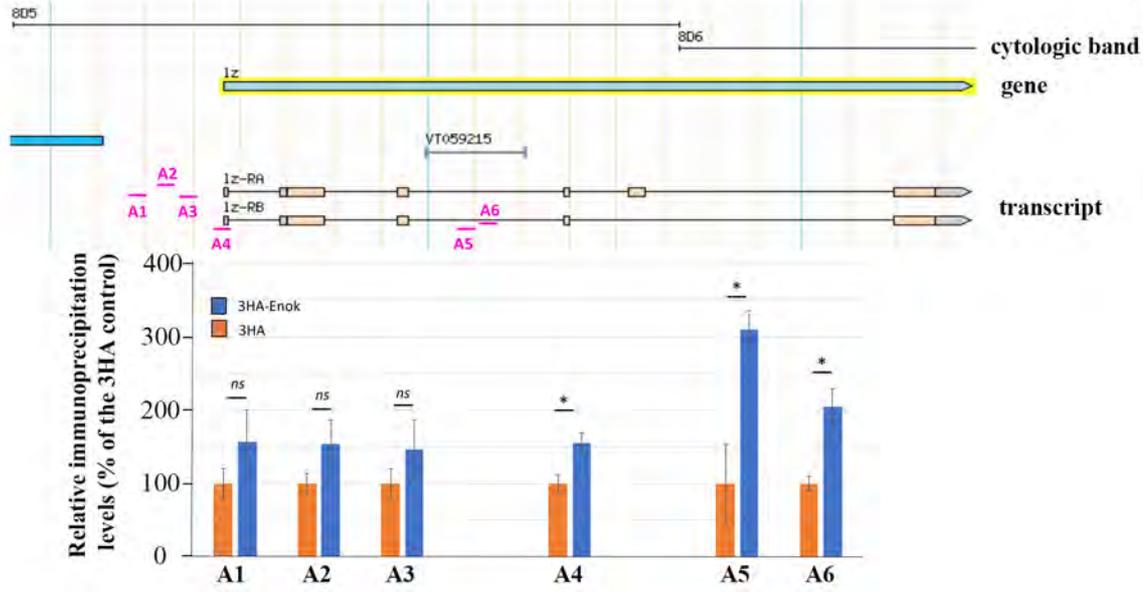
E



F



G



allele, see methods). Despite the critical roles of *lz* during eye development (Daga *et al*, 1996), the excision of *lz* third intron yields individuals with normal eyes, indicating that in these mutants, *lz* expression in the eye imaginal disc is not affected and that the Lz protein is functional. However, like *enok* loss-of-function, *lz^{int3}* mutation abolishes Lz expression in most Notch-activated hemocytes (Fig. 5C,D), indicating that *lz* third intron contributes to its expression in circulating crystal cell precursors. In addition, the *lz^{VT059215}-Gal4* driven re-expression of *lz* in a *lz^{R1}* mutant background is sufficient to restore a wild-type proportion of Lz expressing cells in the NRE-GFP domain (Fig. 5E), as well as crystal cell maturation in the hematopoietic pockets (Fig. 5F). Altogether, these results show that *lz* third intron contains a hematopoietic enhancer both necessary and sufficient for its expression in most crystal cell precursors. We then speculated that Enok might occupy this region to regulate Lz expression in Notch-activated hemocytes. Chromatin immunoprecipitation performed in *tub-Gal4/UAS-3HA-Enok* embryos revealed a conspicuous enrichment in HA-Enok binding across regions of *lz* third intron, corresponding to the identified VT059215 regulatory element (Fig. 5G), and a modest (yet statistically significant) binding of HA-Enok in *lz* first exon, which was recently reported to be bound by Br140 (Kang *et al*, 2017), suggesting potential co-occupancy of this region by the Enok/Br140 complex. No obvious enrichment was detected in the upstream intergenic region previously reported to contain some *lz* regulatory elements (Bataillé *et al*, 2005; Ferjoux *et al*, 2007). Overall, our results point to the VT059215 region as a major site of Enok binding in the *lz* gene, and we propose that Enok regulates *lz* expression by directly binding a hematopoietic enhancer located within its third intronic region.

4. Discussion

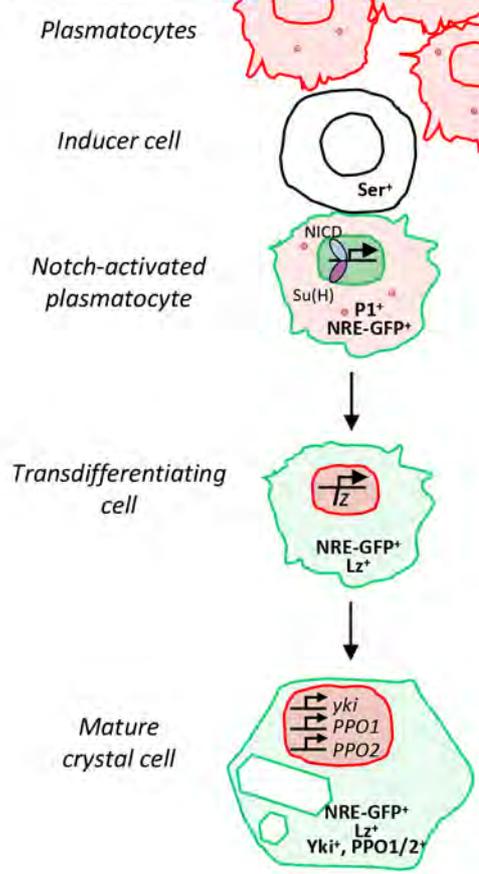
In the present study, we characterized the hematopoietic defects induced by the loss of *enok* function and demonstrated that it is required in a cell-autonomous manner in Notch-activated hemocytes during the transdifferentiation of plasmatocytes into crystal cells that occurs in the hematopoietic pockets. In particular, we presented evidence that *enok* acts downstream of Notch signaling pathway to ensure expression of the crystal cell fate determinant Lz in crystal cell precursors (summarized in Fig. 6). In addition, we showed that this function of Enok relies on a KAT-independent mechanism, presumably by binding the new hematopoietic enhancer we identified within the third intron of *lz*.

One remarkable feature of this enhancer is that it does not allow faithful recapitulation of the Lz expression pattern in Notch-activated crystal cell precursors. Indeed, only a fraction (about 65%) of the NREGFP⁺/Lz^{VT059215}-RedStinger⁺ cells express endogenous Lz, and a large proportion (almost 30%) of the NREGFP⁺/Lz⁺ cells do not express the *lz*^{VT059215}-RedStinger reporter construct. One explanation for this behavior is that the *lz*^{VT059215} enhancer might be incomplete; as a matter of fact, it corresponds to a sequence of only 2 kb in the third intron, whose size reaches 5 kb in total. However, our analysis of the *lz*^{GMR26G10}-Gal4 and *lz*^{GMR25H03}-Gal4 transgenic lines, which contain regulatory regions overlapping with the *lz*^{VT059215} sequence and allow coverage of the full intron length, did not reveal any additional transcription-promoting elements. Another possibility is that the hematopoietic enhancer located in the third intron of *lz* and bound by Enok is not the sole regulatory region controlling its expression in crystal cell precursors. This hypothesis is further supported by the fact that *lz*^{int3} mutation leads only to a partial defect in larval crystal cell formation, since approximately 20% of the NRE-GFP⁺ circulating cells express Lz normally, despite the excision of the whole intron. A second regulatory region would therefore be responsible for the expression of Lz in the 20% of cells that are still able to differentiate properly following Notch signaling activation in *lz*^{int3} mutant individuals. Importantly, loss of *enok* function shares exactly the same partial phenotype as excision of the third intron of *lz*. One would therefore expect the postulated second regulatory region not to be under the influence of *enok*. One very obvious candidate for this additional regulatory element role is the 1.5 kb long *lz*-upstream region, which was shown to recapitulate Lz hematopoietic expression during embryogenesis (Bataillé *et al*, 2005;

Figure 6. Model of stepwise plasmatocyte to crystal cell transdifferentiation in larval Hematopoietic Pockets. We hypothesize that in the sessile microenvironment, similar to what has been described in the lymph gland, a Serrate-expressing inducer cell activates the Notch signaling pathway in a neighboring plasmatocyte. This activated plasmatocyte (crystal cell precursor) responds by expressing the NRE-GFP reporter gene. Notch pathway activation then results in the expression of the crystal cell fate determinant *Lz*, which in turn ensures downregulation of the plasmatocyte specific marker *P1* and promotes expression of *yki* as well as that of the *PPO1* and *PPO2* terminal differentiation markers.

Figure 6

**Hematopoietic Pockets
(sessile hemocytes)**



Ferjoux *et al*, 2007) and appears not to be bound by Enok in our chromatin immunoprecipitation. With the discovery of a novel enhancer contributing specifically to Lz expression in the circulating crystal cell precursors, possibly with the help of another regulatory region, the control of *lozenge* expression appears even more complex than previously thought.

Finally, perhaps our most striking result is that the control of Lz expression relies on a catalytic-independent mode of action of Enok likely involving its interactor Br140, but for which Eaf6 and Ing5 appear to be dispensable. Catalytic-independent functions of MOZ/KAT6A, the mammalian homolog of Enok, have been speculated based on the phenotypic differences observed between mice either totally deprived of MOZ, or in which its catalytic activity has been obliterated by a single amino acid substitution. However, there is so far no documented study of such KAT-independent functions of MOZ *in vivo*. Indeed, although it has been shown that MOZ potentiates RUNX1-dependent transcription independently of its catalytic domain *ex vivo*, this transcriptional co-activator role of MOZ has not yet been reported to occur during any developmental process. During *Drosophila* transdifferentiation of plasmatocytes into crystal cells, we propose that the non-canonic mode of action of Enok relies on its ability to bind DNA. Since Lz is the homolog of mammalian RUNX1 and is known to participate in an autoregulatory loop during embryogenesis (Ferjoux *et al*, 2007), a simple mechanistic hypothesis would be that an Enok/Lz interaction potentiates transcriptional autoregulation of *lz* independently of the catalytic activity of Enok. Alternatively, since MOZ has been shown to interact with a plethora of transcription factors (RUNX1, PU.1, c-JUN, P53, TEL, NF- κ B, NRF2, reviewed in (Perez-Campo *et al*, 2013)) to control expression of various target genes, Enok could also interact with one of their *Drosophila* counterparts, but no such interaction has been reported yet.

More interestingly, it was shown that in *Drosophila* embryos Enok and Br140 interact with Polycomb (Pc), a major epigenetic silencer (Strübbe *et al*, 2011). This was further confirmed in a recent work highlighting the substantial overlap existing between chromatin occupancies by Br140 and by the Polycomb Repressive Complex 1, PRC1 (Kang *et al*, 2017) on developmentally regulated bivalent genes primed for both transcriptional activation and silencing (with activating H3K4me3 and silencing H3K27me3 methylation marks). One of the genomic regions occupied by Br140 and PRC1 is the *lz* locus, and even more strikingly, co-

binding by the BRPF factor BRD1 and the PRC1 member RING1B on the RUNX1-encoding locus was also detected in HUES64 human ES cells. It is noteworthy that the authors did not observe any enrichment in the Enok-dependent H3K23ac mark on their set of bivalent genes, as is it compatible with a non-catalytic mode of action of Enok. The binding of the Enok/Br140 pair, which is rich in epigenetic reader domains, could allow recruitment of other chromatin modifying factors, such as the PRC1 complex, and thereby influence the choice between transcriptional activation or repression in response to a variety of developmental cues.

In conclusion, we have shown that Enok is a major actor of the plasmacyte to crystal cell transdifferentiation during *Drosophila* hematopoiesis. In addition, this study provides the first example of a transcriptional regulation that relies on a non-catalytic mode of action of a KAT6 protein in an integrated model. The regulation of *Iz* expression by an Enok/Br140 complex provides an original paradigm that can be used to address mechanistic questions on a well-defined regulatory element, in a genetically amenable and simple model. Given the large degree of evolutionary conservation of all the identified players, we believe that further elucidation of the non-catalytic mode of action of Enok during *Drosophila* hematopoiesis could be of high relevance to the molecular mechanisms presiding MOZ function in mammalian hematopoietic development, whether normal or malignant.

Supplemental Figure S1. Notch signaling pathway is not globally impaired in an *enok* mutant context. (A) Quantification of the proportions of total circulating hemocytes expressing the NRE-GFP reporter gene in larvae of the following genotypes: *NRE-GFP/+* (control) and *enok¹, NRE-GFP/Df(2R)BSC155* (*enok¹/Df-B155*). (B) Quantification of the proportion of total circulating hemocytes expressing the *UAS-GFP* construct under the control of the *Su(H)-GBE-Gal4* driver. Complete genotypes: *UAS-GFP/+; Su(H)-GBE-Gal4/+* (control) and *UAS-GFP/+; enok¹/Su(H)-GBE-Gal4, Df(2R)BSC155* (*enok¹/Df-B155*). (C) Immunofluorescent staining on circulating cells from larvae of the indicated genotypes; cells were stained with DAPI and α -Lz antibody. Complete genotypes: *E(spl)m β -GFP/+* (control) and *E(spl)m β -GFP, enok¹/Df(2R)BSC155* (*enok¹/Df-B155*). (A,B) Each dot represents the proportion observed in an individual larva. Statistics: ** indicate a p-value ≤ 0.0100 ; ns: not significantly different from reference sample.

Supplemental Figure S2. *Notch^{GMR30A01}-Gal4* directs expression in the crystal cell lineage. (A) Proportion of NRE-GFP⁺ cells expressing the *Notch^{GMR30A01}-Gal4* construct or not, in *NRE-GFP/+; Notch^{GMR30A01}-Gal4, UAS-RedStinger* individuals. Each dot represents the proportion observed in an individual larva. (B) Lineage-tracing of the *Notch^{GMR30A01}-Gal4* driver. Circulating cells were stained with DAPI and α -Lz antibody. Proportions of the indicated cell categories were quantified in a *UAS-RedStinger, UAS-FLP, Ubi-p63E(FRT.STOP)Stinger/Notch^{GMR30A01}-Gal4* genetic context. (RT+Lin): cells positive for either Stinger (lineage-traced, lin) or RedStinger (Real Time expression, RT). *Notch^{GMR30A01}*-unrelated cells have never expressed the driver construct.

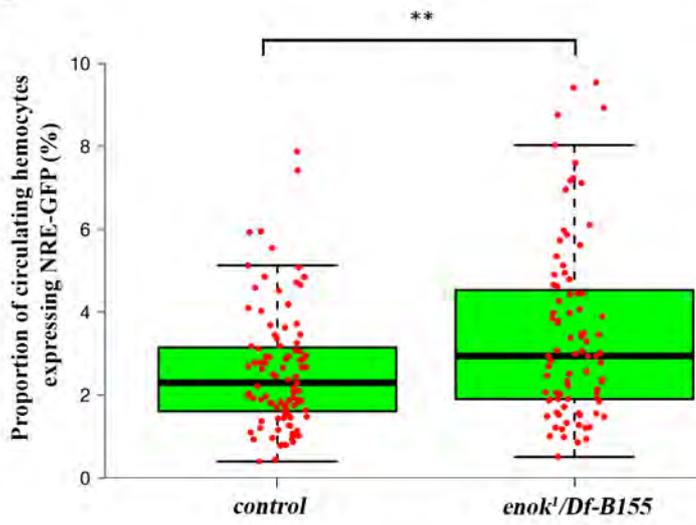
Supplemental Figure S3. H3K23 acetylation in circulating hemocytes is dependent on the ING5 complex and on the catalytic activity of Enok. Mean fluorescence intensity in larval circulating hemocytes (relative to the mean intensity measured in total hemocytes of control larvae), as revealed by an α -acetylated-H3K23 immunostaining. Complete genotypes: *w¹¹¹⁸/+* (control), *enok¹/Df(2R)BSC155* (*enok¹/Df-B155*), *Br140^{S781}/Df(2R)BSC263* (*Br140^{S781}/Df-B263*), *Eaf6^{M26}/Df(3L)BSC387* (*Eaf6^{M26}/Df-B387*), *Ing5^{ex1}/Ing5^{ex1}* (*Ing5^{ex1}*) and *enok^{KAT}/Df(2R)BSC155*

(*enok*^{KAT}/Df-B155). Each dot represents the value attributed to a single cell. Statistics: control genotype was used as a reference sample; *** indicate a p-value≤0.0010.

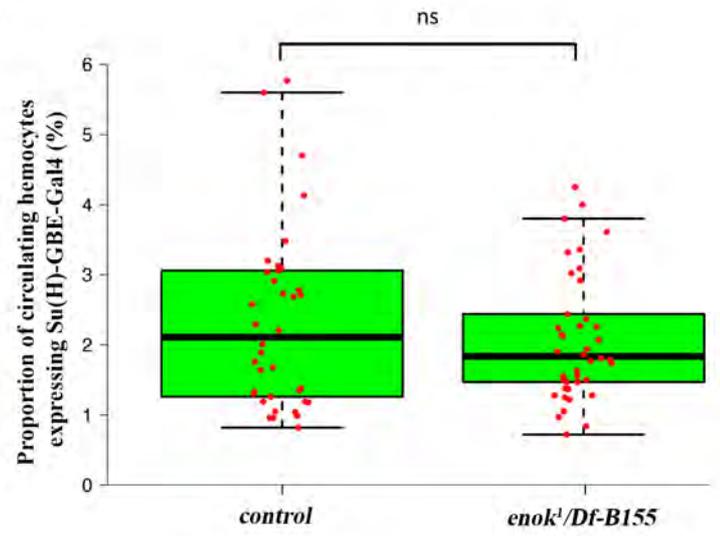
Table 1: Expression pattern of *lz*-related Vienna Tile and Janelia lines

Figure S1

A



B



C

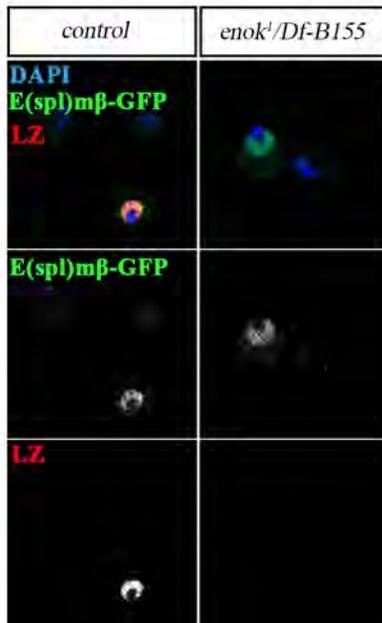
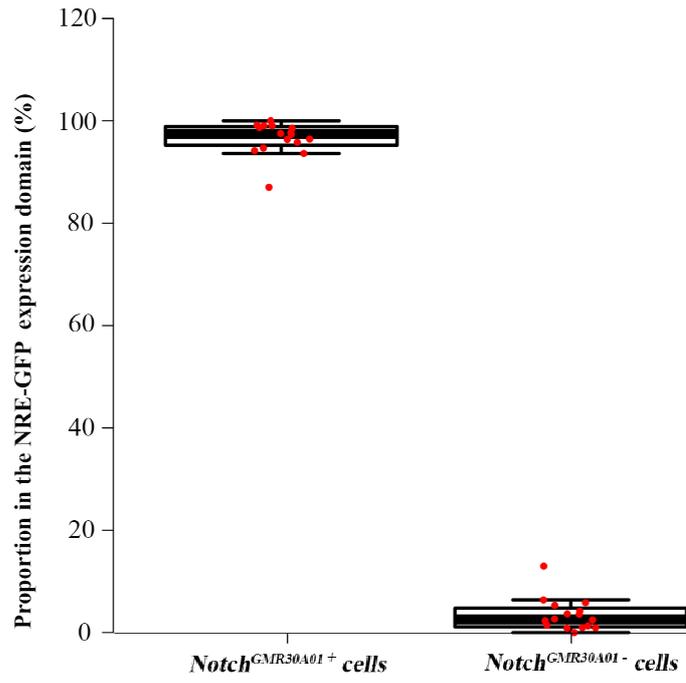


Figure S2

A



B

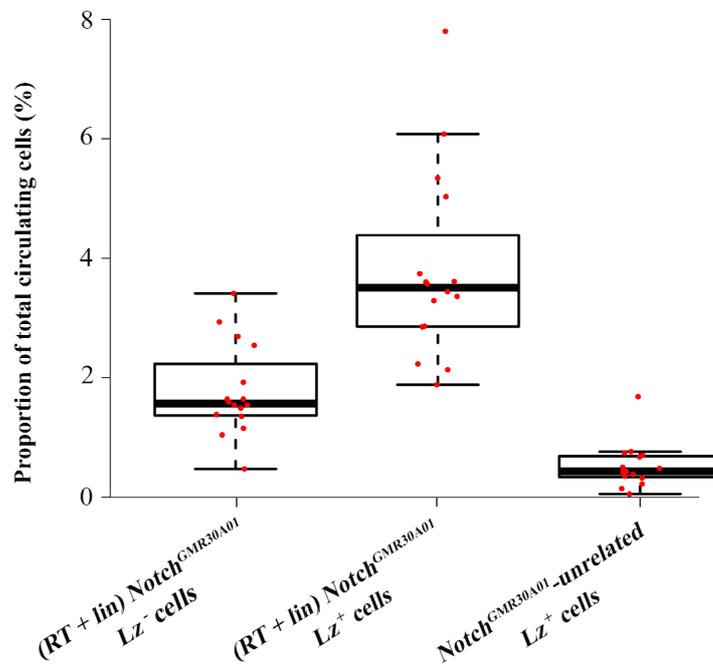
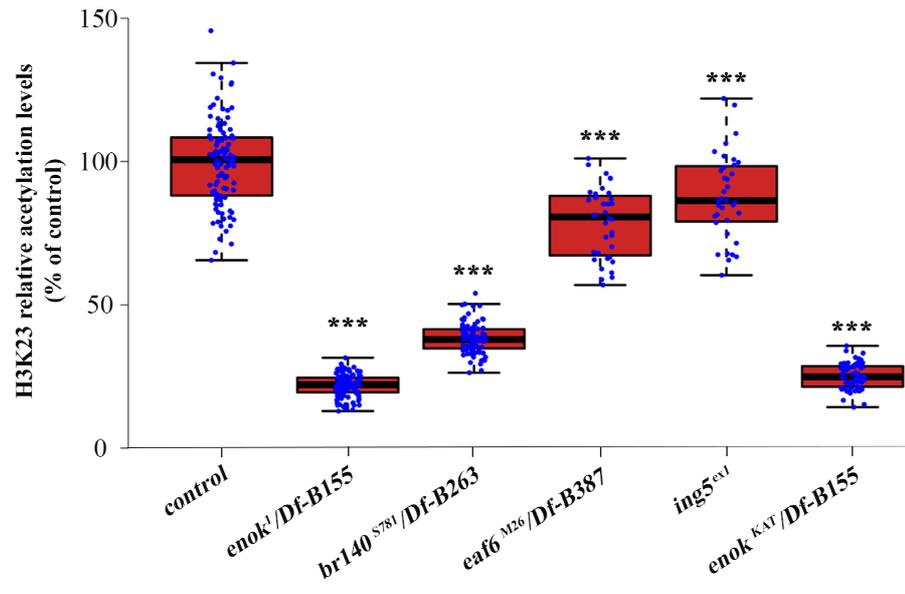


Figure S3

A



Vienna Tile Ref.	VDRC stock num	Red-Stinger expressed in NRE-GFP+ cells ?
VT059212	v208100	no
VT059213	v205984	no
VT059215	v214287	expressed in a subset of NRE-GFP+ cells
VT059219	v207032	no
VT059220	v206996	expressed in a large fraction of circulating cells, independently of the NRE-GFP reporter gene
VT059221	v213815	no
VT059222	v215022	no
VT059223	v203230	no
VT059224	v201848	no
VT059225	v203363	no
VT059226	v205681	no
VT059228	v201301	no
VT059229	v202842	no
GMR26G10	BL48067	no
GMR25H03	BL49143	no
GMR25F01	BL49127	no
GMR26A07	BL46172	no

Table 1: Expression pattern of *Iz*-related Vienna Tile and Janelia lines

7. Acknowledgments

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8. References

- Banerjee U, Girard JR, Goins LM & Spratford CM (2019) *Drosophila* as a Genetic Model for Hematopoiesis. *Genetics* **211**: 367–417
- Barolo S, Castro B & Posakony JW (2004) New *Drosophila* transgenic reporters: insulated P-element vectors expressing fast-maturing RFP. *BioTechniques* **36**: 436–440, 442
- Bataillé L, Augé B, Ferjoux G, Haenlin M & Waltzer L (2005) Resolving embryonic blood cell fate choice in *Drosophila*: interplay of GCM and RUNX factors. *Development* **132**: 4635–4644
- Berger J, Senti K-A, Senti G, Newsome TP, Asling B, Dickson BJ & Suzuki T (2008) Systematic identification of genes that regulate neuronal wiring in the *Drosophila* visual system. *PLoS Genet.* **4**: e1000085
- Bischof J, Björklund M, Furger E, Schertel C, Taipale J & Basler K (2013) A versatile platform for creating a comprehensive UAS-ORFeome library in *Drosophila*. *Development* **140**: 2434–2442
- Daga A, Karlovich CA, Dumstrei K & Banerjee U (1996) Patterning of cells in the *Drosophila* eye by Lozenge, which shares homologous domains with AML1. *Genes Dev.* **10**: 1194–1205
- Doyon Y, Cayrou C, Ullah M, Landry A-J, Côté V, Selleck W, Lane WS, Tan S, Yang X-J & Côté J (2006) ING tumor suppressor proteins are critical regulators of chromatin acetylation required for genome expression and perpetuation. *Mol. Cell* **21**: 51–64
- Dreveny I, Deeves SE, Fulton J, Yue B, Messmer M, Bhattacharya A, Collins HM & Heery DM (2014) The double PHD finger domain of MOZ/MYST3 induces α -helical structure of the histone H3 tail to facilitate acetylation and methylation sampling and modification. *Nucleic Acids Res.* **42**: 822–835
- Ferguson GB & Martinez-Agosto JA (2014a) Kicking it up a Notch for the best in show: Scalloped leads Yorkie into the haematopoietic arena. *Fly (Austin)* **8**: 206–217
- Ferguson GB & Martinez-Agosto JA (2014b) Yorkie and Scalloped signaling regulates Notch-dependent lineage specification during *Drosophila* hematopoiesis. *Curr. Biol.* **24**: 2665–2672
- Ferjoux G, Augé B, Boyer K, Haenlin M & Waltzer L (2007) A GATA/RUNX cis-regulatory

module couples *Drosophila* blood cell commitment and differentiation into crystal cells. *Dev. Biol.* **305**: 726–734

Fujiwara T (2017) GATA Transcription Factors: Basic Principles and Related Human Disorders. *Tohoku J. Exp. Med.* **242**: 83–91

Gajewski KM, Sorrentino RP, Lee JH, Zhang Q, Russell M & Schulz RA (2007) Identification of a crystal cell-specific enhancer of the black cells prophenoloxidase gene in *Drosophila*. *Genesis* **45**: 200–207

Holbert MA, Sikorski T, Carten J, Snowflack D, Hodawadekar S & Marmorstein R (2007) The human monocytic leukemia zinc finger histone acetyltransferase domain contains DNA-binding activity implicated in chromatin targeting. *J. Biol. Chem.* **282**: 36603–36613

Holz A, Bossinger B, Strasser T, Janning W & Klapper R (2003) The two origins of hemocytes in *Drosophila*. *Development* **130**: 4955–4962

Hu D & Shilatifard A (2016) Epigenetics of hematopoiesis and hematological malignancies. *Genes Dev* **30**: 2021–2041

Huang F, Paulson A, Dutta A, Venkatesh S, Smolle M, Abmayr SM & Workman JL (2014) Histone acetyltransferase Enok regulates oocyte polarization by promoting expression of the actin nucleation factor spire. *Genes Dev.* **28**: 2750–2763

Huang F, Saraf A, Florens L, Kusch T, Swanson SK, Szerszen LT, Li G, Dutta A, Washburn MP, Abmayr SM & Workman JL (2016) The Enok acetyltransferase complex interacts with Elg1 and negatively regulates PCNA unloading to promote the G1/S transition. *Genes Dev.* **30**: 1198–1210

Kang H, Jung YL, McElroy KA, Zee BM, Wallace HA, Woolnough JL, Park PJ & Kuroda MI (2017) Bivalent complexes of PRC1 with orthologs of BRD4 and MOZ/MORF target developmental genes in *Drosophila*. *Genes Dev.* **31**: 1988–2002

Katsumoto T, Aikawa Y, Iwama A, Ueda S, Ichikawa H, Ochiya T & Kitabayashi I (2006) MOZ is essential for maintenance of hematopoietic stem cells. *Genes Dev.* **20**: 1321–1330

Katsumoto T, Yoshida N & Kitabayashi I (2008) Roles of the histone acetyltransferase monocytic leukemia zinc finger protein in normal and malignant hematopoiesis. *Cancer Sci.* **99**: 1523–1527

Kitabayashi I, Aikawa Y, Nguyen LA, Yokoyama A & Ohki M (2001) Activation of AML1-mediated transcription by MOZ and inhibition by the MOZ-CBP fusion protein. *EMBO J.* **20**:

7184–7196

Lam K & Zhang D-E (2012) RUNX1 and RUNX1-ETO: roles in hematopoiesis and leukemogenesis. *Front Biosci (Landmark Ed)* **17**: 1120–1139

Leitão AB & Sucena É (2015) *Drosophila* sessile hemocyte clusters are true hematopoietic tissues that regulate larval blood cell differentiation. *Elife* **4**:

Letourneau M, Lapraz F, Sharma A, Vanzo N, Waltzer L & Crozatier M (2016) *Drosophila* hematopoiesis under normal conditions and in response to immune stress. *FEBS Lett.* **590**: 4034–4051

Loubiere V, Delest A, Schuettengruber B, Martinez A-M & Cavalli G (2017) Chromatin Immunoprecipitation Experiments from Whole *Drosophila* Embryos or Larval Imaginal Discs. *Bio-protocol* **7**: e2327

Lv D, Jia F, Hou Y, Sang Y, Alvarez AA, Zhang W, Gao W-Q, Hu B, Cheng S-Y, Ge J, Li Y & Feng H (2017) Histone acetyltransferase KAT6A upregulates PI3K/AKT signaling through TRIM24 binding. *Cancer Res* **77**: 6190–6201

Makhijani K & Brückner K (2012) Of blood cells and the nervous system: hematopoiesis in the *Drosophila* larva. *Fly (Austin)* **6**: 254–260

Menegatti S, de Kruijf M, Garcia-Alegria E, Lacaud G & Kouskoff V (2019) Transcriptional control of blood cell emergence. *FEBS Lett.*

Miller M, Chen A, Gobert V, Augé B, Beau M, Burlet-Schiltz O, Haenlin M & Waltzer L (2017) Control of RUNX-induced repression of Notch signaling by MLF and its partner DnaJ-1 during *Drosophila* hematopoiesis. *PLoS Genet.* **13**: e1006932

Milton CC, Grusche FA, Degoutin JL, Yu E, Dai Q, Lai EC & Harvey KF (2014) The Hippo Pathway Regulates Hematopoiesis in *Drosophila melanogaster*. *Curr. Biol.* **24**: 2673–2680

Perez-Campo FM, Borrow J, Kouskoff V & Lacaud G (2009) The histone acetyltransferase activity of monocytic leukemia zinc finger is critical for the proliferation of hematopoietic precursors. *Blood* **113**: 4866–4874

Perez-Campo FM, Costa G, Lie-a-Ling M, Kouskoff V & Lacaud G (2013) The MYSTerious MOZ, a histone acetyltransferase with a key role in haematopoiesis. *Immunology* **139**: 161–165

Qiu Y, Liu L, Zhao C, Han C, Li F, Zhang J, Wang Y, Li G, Mei Y, Wu M, Wu J & Shi Y (2012) Combinatorial readout of unmodified H3R2 and acetylated H3K14 by the tandem PHD finger

of MOZ reveals a regulatory mechanism for HOXA9 transcription. *Genes Dev.* **26**: 1376–1391

Scott EK, Lee T & Luo L (2001) enok encodes a *Drosophila* putative histone acetyltransferase required for mushroom body neuroblast proliferation. *Curr. Biol.* **11**: 99–104

Strübbe G, Popp C, Schmidt A, Pauli A, Ringrose L, Beisel C & Paro R (2011) Polycomb purification by in vivo biotinylation tagging reveals cohesin and Trithorax group proteins as interaction partners. *Proc. Natl. Acad. Sci. U.S.A.* **108**: 5572–5577

Terriente-Felix A, Li J, Collins S, Mulligan A, Reekie I, Bernard F, Krejci A & Bray S (2013) Notch cooperates with Lozenge/Runx to lock haemocytes into a differentiation programme. *Development* **140**: 926–937

Ullah M, Pelletier N, Xiao L, Zhao SP, Wang K, Degerny C, Tahmasebi S, Cayrou C, Doyon Y, Goh S-L, Champagne N, Côté J & Yang X-J (2008) Molecular architecture of quartet MOZ/MORF histone acetyltransferase complexes. *Mol. Cell. Biol.* **28**: 6828–6843

Voss AK, Collin C, Dixon MP & Thomas T (2009) Moz and retinoic acid coordinately regulate H3K9 acetylation, Hox gene expression, and segment identity. *Dev. Cell* **17**: 674–686

Waltzer L, Ferjoux G, Bataillé L & Haenlin M (2003) Cooperation between the GATA and RUNX factors Serpent and Lozenge during *Drosophila* hematopoiesis. *EMBO J.* **22**: 6516–6525

Xin T, Xuan T, Tan J, Li M, Zhao G & Li M (2013) The *Drosophila* putative histone acetyltransferase Enok maintains female germline stem cells through regulating Bruno and the niche. *Dev. Biol.* **384**: 1–12

B. Complementary results

1. Complements on the study of Enok function during crystal cell formation

As previously stated, during larval life, the catalytic activity of Enok is not required in circulating hemocytes for plasmatocyte to crystal cell differentiation. In order to gain further mechanistic insights about its function during this process, I initiated a structure-function study of Enok using the CRISPR/Cas9-based genome editing method.

Engineering alleles mutant for the DNA binding properties of the MYST domain

It has been shown *in vitro* that MOZ is able to directly bind chromatin and thus has a DNA binding function (Holbert et al. 2007). In this study the authors show that the I727E mutation drastically affects the DNA-binding ability of MOZ, without affecting its capacity to acetylate histones. A comparison of MOZ and Enok amino acid sequences reveals that the residues involved in this binding are conserved in Enok (Figure 11A), and thus I decided to mimic the I727E mutation in *Drosophila*. Using the CRISPR/Cas9 technique, I successfully introduced the I931E desired mutation (Figure 11A), but a melanization assay on *enok*^{I931E} mutant larvae showed no obvious defect in the ability to generate larval crystal cells. Furthermore, the mutation did not affect fly viability. These preliminary observations suggest that the mutation does not affect Enok's DNA binding ability, or that DNA-binding is not mandatory for crystal cell differentiation. I obtained this mutant fly strain late during my doctoral training and thus didn't have time to study it in depth; in order to draw a conclusion, this mutant allele has to be further characterized. In particular, it would be interesting to test its ability to bind the enhancer located in the third intron of *Iz*, but this would require generating additional tools; one could mutate the UAS-3HA-enok construct I used previously for Chromatin Immunoprecipitation, (see section IV.A.3), or one could directly tag the endogenous *enok*^{I931E} protein to realize the same kind of experiment.

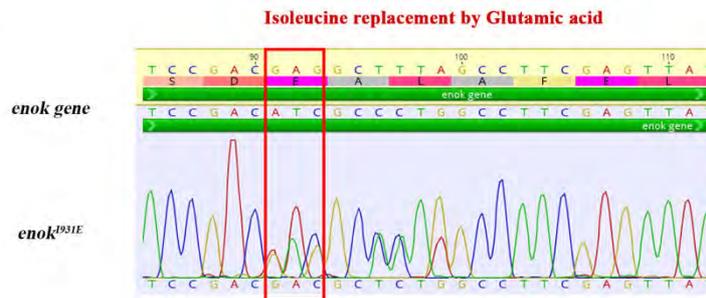
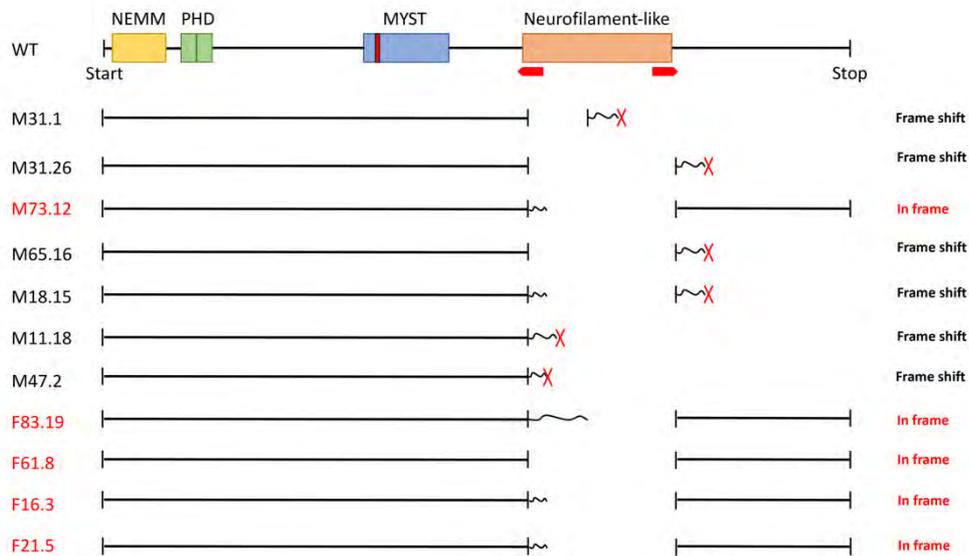
A**B**

Figure 11. Structure function study of the Enok protein

A. In the lower panel is represented the result for the I931E mutation which aims at disabling DNA binding ability of Enok. The sequenced individual was heterozygous, which results in double peaks at the site of interest corresponding to the modified and wild type alleles. Additional silent mutations were introduced for screening purposes. **B.** Top: schematic representation of the Enok protein. Here are represented at scale the N-terminal part of Enok MOZ MORF (NEMM), the tandem Plant Homeodomains (PHD), the MYST and Neurofilament-like domains. Red arrows represent the location of the sgRNA used for the directed excision. Bottom: representation of all mutant alleles recovered during the screen. Left: allele names / Right: result on translated proteins. In frame deletions are represented in red, and new stop codons by red crosses.

Engineering mutants carrying an excision of the Neurofilament domain

In addition to the MYST domain, there are a few identified domains in Enok, and the largest one is called the Neurofilament-like domain. Its function is not described in the literature and thus, using CRISPR/Cas9-mediated deletions, I undertook to excise the Neurofilament-like domain and look at its requirement for larval crystal cell formation (Figure 11B). Two of these deletions will be described below. In order to assess the role of the Neurofilament-like domain, I needed to generate a precise in-frame deletion and recovered the *enok*^{F61.8} allele, whose sequence corresponds to the desired modification. I also included the *enok*^{M31.1} allele in my analysis, because this deletion generates a stop codon after the MYST domain and removes the entire C-terminal part of the protein. Analysis of the established stocks showed that the *enok*^{F61.8} allele is homozygous viable, whereas the *enok*^{M31.1} allele is lethal, as is the case for all other *enok* alleles (Scott, Lee, et Luo 2001; Berger et al. 2008). Quantification of the H3K23 acetylation levels in these two new mutant contexts showed that neither excision of the Neurofilament-like domain, nor truncation of the C-terminus of Enok, affects its catalytic activity, since circulating hemocytes exhibit a wild-type level of acetylated H3K23 (Figure 12A). I then quantified the proportion of Lz-expressing cells in circulating crystal cell precursors. I observed that *enok*^{F61.8} and *enok*^{M31.1} alleles displayed a wild-type proportion of differentiating crystal cells (Figure 12B). Altogether these results indicate that the Neurofilament-like domain is not required for *Drosophila* viability, for the catalytic activity of Enok, or for crystal cell development during the larval stages.

The *enok*^{M31.1} allele does not impact the ability of the protein to acetylate its substrates, and homozygous individuals display pupal lethality; together with my previous observations that the catalytic activity of Enok is dispensable for viability, these results suggest that a domain located in the most C-terminal part of Enok is essential for viability. I therefore examined more in detail the sequences corresponding to the C-terminal parts of MOZ and Enok, seeking a domain whose loss would be responsible for the lethality of *enok*^{M31.1} in mutant pupae. I identified some sequence homology between the C-terminal part of Enok and the Proline-rich domains of MOZ (Figure 5), which contain p53 interaction domains that are

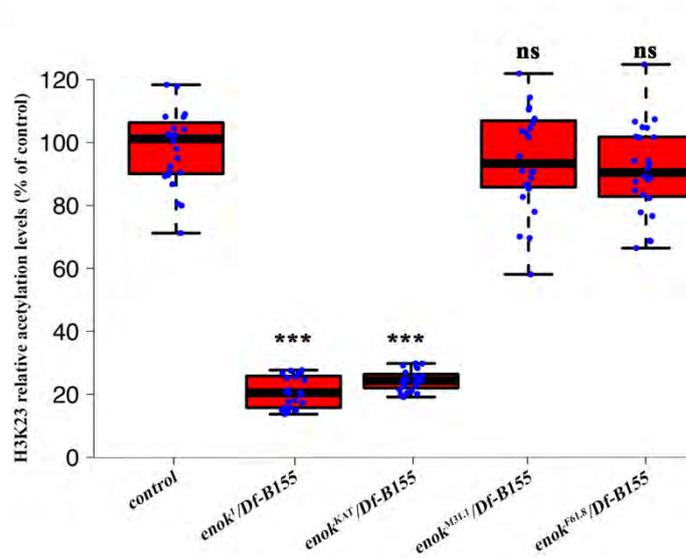
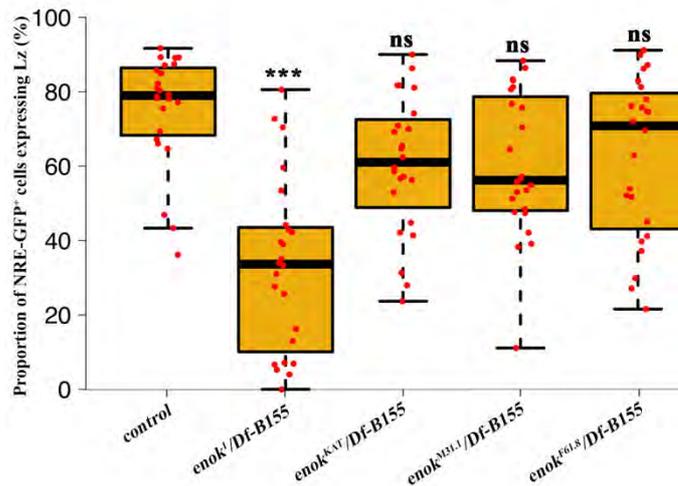
A**B**

Figure 12. The C-terminal half of Enok is not involved in crystal cell formation

A. Quantification of fluorescence intensity after a H3K23ac immunostaining, in various *enok* mutant conditions. *enok¹* and *enok^{KAT}* mutants display a drastic loss of H3K23ac staining, whereas *enok^{F61.8}* and *enok^{M31.1}* mutants do not display any difference with the control condition **B.** Quantification of Notch-activated crystal cell precursors expressing Lz in various *enok* mutant conditions. The *enok¹* mutant shows a strong reduction in Lz-expressing cells proportion, whereas *enok^{KAT}*, *enok^{F61.8}* and *enok^{M31.1}* do not differ from the control condition. Complete genotypes are: *w¹¹¹⁸* (control), *enok¹,NRE-GFP/Df(2R)BSC155* (*enok¹/Df-B155*), *enok^{KAT},NRE-GFP/Df(2R)BSC155* (*enok^{KAT}/Df-B155*), *enok^{M31.1},NRE-GFP/Df(2R)BSC155* (*enok^{M31.1}/Df-B155*), *enok^{F61.8},NRE-GFP/Df(2R)BSC155* (*enok^{F61.8}/Df-B155*).

essential for cell cycle regulation and are involved in tumorigenesis (Susumu Rokudai et al. 2009, 53; S. Rokudai et al. 2013, 53). Furthermore, human mutations introducing a stop codon after the MYST domain lead to microcephaly and overall developmental delay (Tham et al. 2015). In this study the authors linked the developmental disorders to a missing interaction between MOZ and p53, as they saw differential regulation of p53 target genes in these individuals. Interestingly, it has been shown that BRPF1 mutants in human also display intellectual disability (Yan et al. 2017), which would raise the question of its interaction with MOZ and p53 during development. All this evidence led me to postulate the conservation of the interaction between Enok and p53, and that *enok*^{M31.1} mutant allele I had generated could misregulate potentially conserved Enok/p53 target genes, which would in part explain the lethality in the flies.

This hypothesis has to be investigated from the start, because it has never been shown in *Drosophila* that Enok and p53 interact; furthermore, it would be interesting to study the mechanisms underlying *enok*^{M31.1}-related lethality, as the molecular mechanisms involved in this phenotype may be conserved in humans. The conservation of the MOZ/BRPF interaction in *Drosophila* makes it a relevant model with which to study this potential catalytic independent function of MOZ during mammalian development.

2. Complements on the study of the regulatory regions controlling *lz* expression in the crystal cell precursors

Engineering mutants carrying an excision of the lz-upstream region

As discussed in section IV.A.4, the deletion of *lz* third intron is not sufficient to completely abrogate its expression in circulating cells. Indeed, in *lz*^{int3} mutant larvae, around 20% of NRE-GFP positive cells still have the ability to normally initiate *lz* expression, and this phenotype is shared by *enok* loss of function. This observation suggests that there is another regulatory region responsible for *lz* initiation independent of *enok* during plasmatocyte transdifferentiation. The region located upstream of the *lz* gene was shown to recapitulate *lz* hematopoietic expression in the embryo (Bataillé et al. 2005; Ferjoux et al. 2007), and it was

not bound by Enok (see section IV.A.3). I therefore generated a CRISPR-Cas9 mediated excision of this region, the *lz*^{2AB} allele, in order to assess its requirement during crystal cell formation and started to characterize its mutant phenotypes. As for the *lz*^{int3} allele, there was no eye phenotype in the adult fly, indicating that this region was not necessary for the control of *lz* expression in the eye imaginal disc. I observed that *Lz* expression is lost in circulating crystal cell precursors (Figure 13), which suggests that the *lz*^{2AB} region is responsible, at least partially, for *lz* expression during plasmatocyte to crystal cell transdifferentiation.

To resume, we identified two enhancers that direct normal expression of *lz* in Notch-activated hemocytes. It would be interesting to assess the consequences of the simultaneous excision of both regulatory regions. Since the two regulatory regions seem to be partially involved in *lz* expression, deletion of both at the same time might completely abrogate *lz* expression in NRE-GFP⁺ cells. Likewise, it would be interesting to coexpress the *lz*^{VT059215-} *RedStinger* (which is not expressed in all of the NRE-GFP positive cells) with the *lz-LacZ* reporter gene and compare their additive pattern to endogenous *Lz* expression, in order to see if the combination of the two regions is sufficient for the complete expression domain of *lz* in NRE-GFP expressing cells.

These experiments should give more insights into the mechanisms underlying *lz* regulation in the Hematopoietic Pockets, which is for now not characterized.

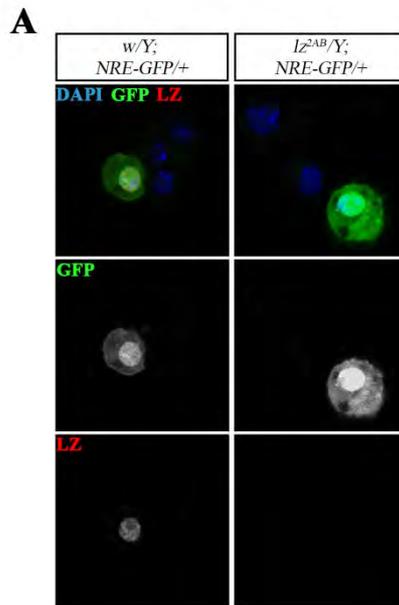


Figure 13. The Iz^{2AB} enhancer is crucial for *Iz* expression in circulating crystal cell precursors.

(A) Immunofluorescent staining on circulating cells from larvae of the indicated genotype; cells were stained with DAPI and α -Lz antibody.

V. Material and methods

***Drosophila* stocks, genetics and melanization assays.**

Stocks and crosses were maintained at 25°C on standard yeast-agar-cornmeal medium. The complete list of stocks used in this study is provided as supplemental material (Table 2). Larvae collected for phenotypic analysis were raised in controlled density conditions: in brief, for each cross, 10 females were fertilized by 4 males and left to lay eggs for 16 hours at 25°C. Third instar wandering larvae of the appropriate genotypes were collected 5 days after egg laying. Female larvae were used, unless otherwise specified. For melanization assays, two batches of six larvae were collected in two 1.5 mL eppendorf tubes containing 200 μ L of Phosphate Buffered Saline (PBS) each, incubated in a 65°C waterbath for 30 minutes, stored on ice for 30 minutes and imaged on a SMZ18 stereomicroscope (Nikon). Each experiment was reproduced at least three times (minimum of total larvae observed for each phenotype, n=36).

Immunofluorescent staining and Operetta quantifications on circulating cells.

Four female third instar larvae were bled in 1ml of PBS in 24-well-plate containing a glass coverslip. Hemocytes were centrifuged for 2 min at 900 g, fixed for 20 minutes with 4% paraformaldehyde in PBS and washed twice in PBS. Cells were then permeabilized in PBS-0.3% Triton (PBST), blocked in PBST-1% Bovine Serum Albumin (BSA) and incubated with primary antibodies at 4°C over night in PBST-BSA. The complete list of primary antibodies used in this study is provided as supplemental material (Table 3). Next, cells were washed in PBST, incubated for 2 hours at room temperature with corresponding Alexa Fluor-labeled secondary antibodies (Molecular Probes), washed in PBST and mounted in Vectashield medium (Eurobio-Vector) following incubation with DAPI. Imaging was performed on a Leica SP8 confocal microscope. For fluorescence quantification, single female larvae were bled individually in 96-well-plate and samples were processed as described above; imaging was performed on an Operetta microscope (Perkin-Elmer); for each well 30 fields were captured at 20X magnification. Statistical analyses were performed with GraphPad Prism (GraphPad Software, Inc.); two-tailed Student t-Test were used for comparisons of two samples and one-way ANOVA followed by Dunnett test for comparisons of more than two samples.

Generation of UAS-3HA-Enok and I^z^{VT059215}-Red-Stinger fly strains.

- *cloning of UAS-3HA-Enok*: a three HA-tags encoding sequence was directionally inserted in the pUASAttB vector (Addgene) polylinker using EcoRI and NotI restriction enzymes, yielding a pUASAttb-3HA plasmid. A p-BlueScript containing the full-length *enok* cDNA (kind gift from Takashi Suzuki) was then used as a template to PCR-amplify the *enok* coding sequence (Phusion high-fidelity polymerase, Thermofisher scientific). The amplified fragment was cloned in frame with the 3HA sequence, between the NotI and KpnI restriction sites of the pUASAttB-3HA vector, using the In-fusion HD cloning kit (Clontech) according to supplier's instructions.

- *cloning of I^z^{VT059215}-Red-Stinger*: this cloning was performed in two steps. We first generated a phiC31-based transformation vector containing a red fluorescent reporter gene compatible with golden gate cloning. Briefly, the pAttB vector (Bischof *et al*, 2013)(kind gift of K Basler) was modified to remove the existing BsaI restriction sites and we then inserted a polylinker containing BsaI restriction sites on each sides of a lacZ gene from the pCambia2200 vector (kind gift of Jean-Philippe Combiere), the hsp70 promoter and the DsRed. T4-NLS coding sequence from the pRed-H-Stinger vector (Barolo *et al*, 2004)(DGRC). In a second step, we cloned the VT059215 sequence into the pAttB-Red-H-Stinger vector with BsaI restriction enzyme following a golden gate cloning protocol.

All constructs were checked by sequencing for polymorphisms/mutations prior to injection for phiC31-mediated insertion in an AttP2 platform containing fly line. All plasmid sequences and detailed cloning procedures are available on request.

Chromatin Immunoprecipitation on embryos.

Four independent chromatin immunoprecipitation experiments were performed as follows: embryos were collected twice a day for 2-3 days, washed, frozen dry and kept at -80°C. They were then processed as described in (Loubiere *et al*, 2017). The immunoprecipitated chromatin was subsequently used in RealTimePCR using the SsoFast Evagreen chemistry (Biorad) in a CFX96 thermocycler (Biorad). 100 mg of embryos were used for each replicate. The complete list of primers used in this study is provided as supplemental material (Table 4).

Generation of mutant fly lines by CRISPR/Cas9-mediated genome editing

Single guide RNA (sgRNA)-compatible sequences were identified in the regions of interest with the Geneious software (Biomatters Ltd); selected sequences had null off-target scores. Oligonucleotides for sgRNA cloning in the pCFD3 expression vector (Port *et al*, 2014) were synthesized by Integrated DNA Technologies and processed according to: <http://www.crisprflydesign.org/wp-content/uploads/2014/05/Cloning-with-pCFD3.pdf>.

Constructs were checked by sequencing prior to injection in *vasa-Cas9* transgenic fly strains (Port *et al*, 2014). Three different editing strategies were used depending on the type of mutant to generate.

- *Eaf6* and *Ing5* genes excisions (*Non Homologous Ends Joining*): we took advantage of the presence of P-elements inserted in the vicinity of the 5'UTR of the genes and used a *mini-white* marker reversion-based screening strategy. Briefly, the d06605 transposon (*Eaf6*) or the EY13664 transposon (*Ing5*) was brought in a *vasa-Cas9* genetic background using standard *Drosophila* genetics. The resulting fly strains were used for injection of two sgRNA-containing pCFD3 vectors targeting each side of the inserted P-element. sgRNA expression vectors were injected at a concentration of 250 ng/uL each. Injected F₀ individuals were crossed, then their F₁ progeny was screened for the reversion of the *mini-white* marker and positive F₁ individuals were used to establish stocks. Gene excisions were confirmed by PCR on genomic DNA extracted from F₂ flies.

- *enok*^{KAT} allele engineering (*single amino acid substitution, Homology Directed Repair*): the K807R mutation introduced in the MYST domain of *enok* affects a Lysine that is highly conserved across evolution and critical for the KAT activity of yeast and human MYST proteins (Yuan *et al*, 2012; Yang *et al*, 2012). A repair DNA template called single stranded oligonucleotide donor (ssODN) was injected along with the sgRNA expression vector so as to direct the reparation (ssODN: 100 ng/uL and sgRNA: 250 ng/uL). The ssODN sequence contained the mutation of interest, as well as a sabotage mutation of the Protospacer Adjacent Motif to avoid re-cleaving of an edited chromosome. In addition, the nucleotide sequence immediately surrounding the desired site of modification was degenerated by introduction of silent mutations, in order to allow the design of a discriminant screening primer. 10-15 F₁ males were crossed individually for each F₀ founder; after 4-5 days in the crossing vials, each F₁ male was retrieved for single fly DNA extraction. The PCR-based screen

of F₁ individuals relied on a triple primer PCR: two external primers amplify DNA in both positive and negative editing events, while an internal primer specifically amplifies DNA if the desired mutation is present.

- *Iz third intron excision (Non Homologous Ends Joining)*: two sgRNA expression vectors targeting each extremity of *Iz* third intron were injected at a concentration of 250 ng/uL each; 10-15 F₁ males were crossed individually for each F₀ founder and after 4-5 days in the crossing vials, they were retrieved and tested for their ability to generate shorter amplicons in PCR reactions.

- *enok^{I931E} allele engineering (single amino acid substitution)*: the mutation was designed to target a conserved aminoacid crucial for MOZ binding to nucleosomes *in vitro* (Holbert et al. 2007). The same injection and screen protocol was used for the generation of the *enok^{KAT}* allele.

- *Neurofilament-like domain excision (Non Homologous Ends Joining)*: two rounds of injections were performed, each using a different pair of sgRNA expression vectors targeting each extremity the domain. The injection concentration was of 250 ng/uL each; F1 individuals were tested for their ability to generate shorter amplicons in PCR reactions.

- *Iz upstream regulatory region excision (Non Homologous Ends Joining)*: two sgRNA expression vectors targeting each extremity of *Iz* third intron were injected at a concentration of 250 ng/uL each; 10-15 F₁ males were crossed individually for each F₀ founder and after 4-5 days in the crossing vials, they were retrieved and tested for their ability to generate shorter amplicons in PCR reactions.

Table 2: Fly stocks list

genotype	nature	origin	reference
w ¹¹¹⁸	spontaneous allele	BL: 3605	Research article: Hazellrigg et al., 1984
enok[1]	EMS-induced loss of function allele	BL: 6284	Research article: Scott et al., 2001
enok[2]	EMS-induced loss of function allele	BL: 6285	Research article: Scott et al., 2001
enok[Q253]	EMS-induced loss of function allele	DGRC: 109887	Research article: Berger et al., 2008
Df(2R)BSC155	FLP/FRT induced deficiency	BL: 9691	Personal communication to Flybase: Christensen, S., Cook, K. (2006.5.11)
NRE-GFP	transgenic insertion	BL: 30727	Personal communication to Flybase: Mendes, G. (2010.2.24)
Su(H)GBE-Gal4	transgenic insertion	kindly provided by S.Hou	Research article: Zeng et al., 2010
E(spl)mBerGFP	transgenic insertion	BL: 65294	Personal communication to Flybase: model organism Encyclopedia of Regulatory Network (modERN) Project, (2015-)
tubulin-Gal4	transgenic insertion	BL: 5138	Research article: Lee et al., 1999
GMR30A01-Gal4	transgenic insertion	BL: 45554	Personal communication to Flybase: Pfeiffer et al., (2011.2.9)
UAS-RedStinger	transgenic insertion	BL: 8545	Personal communication to Flybase: Posakony, J. (2004.6)
UAS-RedStinger, UAS-FLP3, Ubi-p63E(FRT,STOP)Stinged	G-trace stock	BL: 28281	Evans et al., 2009
b ¹⁴⁰ [5781]	EMS-induced loss of function allele	DGRC: 109892	Research article: Berger et al., 2008
Df(2R)BSC263	FLP/FRT induced deficiency	BL: 23162	Personal communication to Flybase: Christensen, S., Cook, K. (2007.3.22)
Df(3L)BSC387	FLP/FRT induced deficiency	BL: 24411	Personal communication to Flybase: Christensen, S., Cook, K., Cook, K. (2007.10.29)
lz[1]	transposon excision induced deficiency	BL: 33834	Research article: Daga et al., 1996
UAS-Lz	transgenic insertion	kindly provided by P.Gergen	Research article: Tracey et al., 1999
UAS-yW-GFP	transgenic insertion	BL: 28815	Research article: Oh et al., 2008
UAS-yW*[S168A]-GFP	transgenic insertion	BL: 28816	Research article: Oh et al., 2008
lzlV1059215]-Gal4	transgenic insertion	VDR C: 214287	https://stockcenter.vdr.caf/control/library_vt
vasa-Cas9	transgenic insertion	BL: 51323	Research article: Port et al., 2014
Q06605	transgenic insertion	BL: 19244	Research article: Thibault et al., 2004
EY13364	transgenic insertion	BL: 20873	Research article: Bellen et al., 2004

antibody raised against	type	source	use	reference
PPO1	rabbit, polyclonal	kind gift of Dr Erjun Ling	immunofluorescent staining	Li et al., 2012
Lz	mouse, monoclonal	Developmental Studies Hybridoma Bank	immunofluorescent staining	Lebestky et al., 2000
P1	mouse, monoclonal	kind gift of Dr istvan Ando	immunofluorescent staining	Kurucz et al., 2007
H3K23ac	rabbit, polyclonal	Merck Millipore (07-355)	immunofluorescent staining	Suka et al., 2001
Yki	rabbit, polyclonal	kind gift of Dr Matthew Gibson	immunofluorescent staining	Ikmi et al., 2014
HA	mouse, monoclonal	Biologend	chromatin immunoprecipitations	-

Table 3 : Antibody stock list

Cloning primers	
3xHANOI-Enok	GCTGCTCATGCGGCCGTATGAGGGAATCGGGCATGATATCAAC
KpnI-STOP-Enok	AAAGATCCTCTAGAGGTACCCGCATCTATTATCTGCGAATAGAACCCTTGAG
VT059215 RedStinger F	CCCGGTCTCCAAATGCACCTGACCTTTTGCCG
VT059215 RedStinger R	CCCGGTCTCCCGTAACTCAATCCCGAAATGTCTTCA
Chromatin Immunoprecipitation primers	
A1 F	GCTGAGTTCTTCTCAGCT
A1 R	TCCGACTTATCACATCGAAGGG
A2 F	TTCGACCACAAACCAAGGCT
A2 R	AGCGAAGGAAAGGCGTGATA
A3 F	AACAGCACTGCGTGGAAATGG
A3 R	GCTCTTCTGTAATGCATGGCC
A4 F	AGTAAACAAAAAGCGCGCA
A4 R	AGTTGGTGACGAAATTCGAGTT
A5 F	GACTTAGTGTGCGCATGGGC
A5 R	TTCCGTGGAAAACCTGCCTGT
A6 F	GGATGCTCCACATTTTGCGG
A6 R	ATAGGCCTGGCTCTTTTCG
CRISPR guide RNA	
guide K807R	CGTCGTAGTAGAGTGTTTTG
guide ING5 5'	CTGTAAGTAATCGATAACT
guide ING5 3'	ATCCACGCGTGCCAAATCCG
guide EAF6 5'	ACTGGCTTCAAATGCAAG
guide EAF6 3'	AGTGCGACAGCGAAGAAAAG
guide IZint3 3'	AGCGGCACGTCATTAGAGAG
guide neuro 1 5'	CATCGGCACCCATTCCCAT
guide neuro 1 3'	GTCCAGCCAAGGAAATAACT
guide neuro 2 5'	AGTTGGATCTTGTCCGAAAT
guide neuro 2 3'	TCCTAGTTATTTCTTGGC
guide I931E	AGTTCAATAACTCGAAGGCC
guide IZint3 5'	GTGCTGTCCGAACGGTCACT
ssODN sequence	
ssODN K807R	ATTTATTGCCAGAACCTGTGCTGTGGCCAAAGTTCTTCTCGACCACAGGACGTTATACTACGACGTAGAACCCTTCTGTTCTATATTCTCACTAAGAATGAT CAAAGCGGGTGTCAATTTGGTTGGCTACTTCTCAAAGGAGAAGCACTGCACCCAAAAGTACAATGTGTCGCTATCCTGACGATGCCGAGTAC
ssODN I931E	GTCGTGTTGGAGTATTTGTACAAGCATCGCAACTACACAAAGATCACCTCAAGGACATCGCCATTAACAGGTCTGGCTATTTCCGACGAGGCTTTAGCCT TCGAGTTATTGAACTTCATCAAGCTAAGAAAGAACGATGGCGACATTAGGTATCAGATTAATGTGAAAATTGAGTGGAAAAGGTTTTGGCCAC
CRISPR genotyping primers	
Geno Ing5 F	TGCCAATTGTTTTGGGTTA
Geno Ing5 R	TCCGAATTAAGGGCATTGA
Geno Eaf6 F	TGACGCGATGCTGAAAGTAG
Geno Eaf6 R	CCACGGGGAGATCATTAGAA
Geno IZint3 F	CGCAACTTACCACCCAGAT
Geno IZint3 R	TGATGGCTTTGGCGTAGGTT
Geno enok F	GGATGGCAATGTCAACAAGA
Geno enok R	TCCAACACGACTGATTTCCA
K807R F	TTCTCGACCACAGGACGTTA
geno I931E F	GCTTCTGATCGACTTCAAGC
geno I931E R	TGGTTGCTCAAATATTCAGAT
I931E F	GCTATTTCCGACGAGGCTTTA
geno ΔNeuro F	TCCGAGGAGCCTCAGTTCA
geno ΔNeuro R	TGCGTCCAGGTGAGAGAGA

Table 4 : Primer and CRISPR reagent list

VI. Discussion

A. General conclusions

During my PhD work I described for the first time that Enok, the *Drosophila* counterpart of MOZ, also plays a critical role during *Drosophila* larval hematopoiesis. Enok is indeed required for the formation of one of the three blood cell lineages, the crystal cell lineage. It controls the expression of *lz*, which encodes a RUNX transcription factor specific of the crystal cell lineage. In addition, I showed that Enok plays its role in a cell-autonomous manner in crystal cell precursors that are plasmatocytes activated by the Notch signaling pathway. Also, I demonstrated that, Enok acts independently of its catalytic activity together with only one of its canonic partners, Br140, to control *lz* expression. Furthermore, I showed that Enok is able to bind specifically the third intron of *lz*, on a genomic region that drives expression in the crystal cell progenitors and, more importantly, which is able to rescue *lz* loss of function phenotype by overexpressing *lz* itself. In addition, excision of this third intron leads to a hematopoietic specific loss of *lz* function, since mutant larvae are deprived of crystal cells in circulation.

In the last part of my training, I generated specific deletions in the *enok* gene using the CRISPR/Cas9 system. While the Neurofilament-like domain of Enok appears dispensable for its functions, deletion of the C-terminus of *enok* induces lethality without affecting hematopoiesis or its ability to acetylate H3K23. This phenotype indicates an essential role of Enok for survival, which relies on its C-terminal part.

Altogether, my results unveil a new mode of action for Enok in a process in which it has never been implicated before. The mode of action that we uncovered may bring new insights into MOZ functions in mammalian development, during which it has been mostly studied considering its catalytic activity. More particularly, my work could lead to a better understanding of MOZ mode of action in both normal and pathological hematopoiesis.

To conclude this manuscript, I will discuss several points that have raised my interest during my PhD work. I showed that *lz* expression in Hematopoietic Pockets is not induced by Yki. Since Yki expression is detected in NRE-GFP positive cells, I will first discuss about a potential role of Yki during crystal cell differentiation. Then, I will discuss about the catalytic

independent roles and mode of action of Enok throughout development and more particularly during hematopoiesis. Finally, I will discuss the possibility that *Drosophila* could be a valuable model to study the molecular mechanisms involved during MOZ-related diseases in mammals.

B. A possible role for Yki in Hematopoietic Pockets

I demonstrated that the regulation of *lz* expression in circulating Notch-activated hemocytes is not the same as what has been shown by Ferguson *et al* in the lymph gland (Ferguson et Martinez-Agosto 2014). Indeed, the Hippo pathway effector Yorkie is not initiating *lz* expression in circulating crystal cell precursors, since in this compartment *lz* expression precedes that of *yki*. There are major differences between the sessile/circulating and lymph gland compartments, and thus crystal cell differentiation is likely to rely on different mechanisms depending on the cellular context. Indeed, it is reported that in the lymph gland crystal cells are formed directly from the *de novo* differentiation of naïve progenitors, whereas in Hematopoietic Pockets crystal cells are generated by the transdifferentiation of mature, fully differentiated plasmacytes (Leitão et Sucena 2015). What I show in my work may therefore be a specific mechanism involved only in the transdifferentiation process that takes place in Hematopoietic Pockets.

In a recent study, Jang *et al* showed that when YAP, the mammalian homolog of Yki, is phosphorylated and sequestered in the cytoplasm, a fraction of phosphorylated YAP remains in the nucleus and can interact with RUNX1 and/or RUNX3 (Jang et al. 2017). It is noteworthy that, while YAP interaction with its canonic partner TEAD promotes proliferation (Hippo pathway off), the interaction between phosphorylated YAP and RUNX3 occurs during cell cycle arrest (Hippo pathway on). Furthermore, the authors showed that *lz* overexpression is able to rescue the eye overgrowth phenotype induced by Yki^{S168A} (the activated form of Yki) overexpression. This experiment showed that *Lz* is able to antagonize Yki activity; this antagonism might rely on a conserved interaction between RUNX factors and YAP. This study suggests that in *Drosophila* *Lz* could interact with Yki to inhibit its proliferative effects. Interestingly, in the hematopoietic pockets plasmacytes are able to proliferate, which is in agreement with the absence of Hippo pathway activation. In these cells, Yki could interact

with Sd in the nucleus to promote proliferative genes expression. After their transdifferentiation into crystal cells, which have never been shown to have any dividing capacities, the Hippo pathway would be turned on. This model fits the observation that Yki is mostly localized in the cytoplasm of crystal cells. Considering that phosphorylated YAP is able to interact with RUNX factors, it is possible to hypothesize that Lz may form a complex with phosphorylated Yki in the crystal cell nucleus to promote the expression of their target genes. It is further interesting to note that Milton *et al* showed that Yki is able to bind directly *lz* third intron (Milton et al. 2014); among all the possible binding regions of a hypothetical Lz/Yki complex, this third intron itself is a good candidate, as it could be required for the maintenance of *lz* expression in differentiating crystal cells.

YAP interacting domain on RUNX1 has been identified, thus if the amino acid sequence homology is sufficient between RUNX1 and Lz, it would be possible to mutate the identified Yki-interacting domain in Lz, and see if Lz/Yki interaction is required for correct *lz* expression during crystal cell differentiation *in vivo*.

C. Study of Enok catalytic-independent activity during *Drosophila* development

1. *enok* KAT activity during embryogenesis

Catalytically inactive *enok* mutants are viable and I observed that homozygous adult females are sterile. This may be due to a requirement of this KAT activity in the germline, as it has been shown that Enok plays an essential role both in the maintenance of germline stem cells (Xin et al. 2013) and oocyte polarization (Huang et al. 2014) in females. Since catalytically inactive mutant females do not display any obvious defect in egg-laying, the sterility is likely due to an embryonic development failure. Huang *et al* demonstrated that *enok* mutant germline clone embryos (lacking maternal contribution) die during embryonic development due to a segmentation defect (Huang et al. 2014). It could be interesting to look at the early stages of development in *enok*^{KAT} mutant embryos to determine if the arrest in embryonic development is caused by the same segmentation defect.

Thanks to the strong *enok* maternal contribution, mutant phenotypes only appear in the larval stages, thus preventing us from characterizing easily a potential function of *enok* in embryonic hematopoiesis. However, *enok* mutant germline clone embryos develop until at least embryonic stage 12. Since *srp* expression is detected in prohemocytes at stage 5, and crystal cells specific markers *PPO1* and *PPO2* are expressed at stage 11, *enok* mutant germline clone embryos can be used to address the question of *enok* function in embryonic hematopoiesis. If hematopoietic defects are observed in germline clone embryos, the comparison with embryos laid by *enok*^{KAT} mutant females should establish whether they depend on the catalytic activity of Enok or not. Alternatively, it could be possible to investigate *enok* function during embryonic hematopoiesis by using targeted RNA interference. In such experiments it would be possible to investigate specifically *enok* involvement at different steps of the hematopoietic process, such as prohemocyte emergence or differentiation.

2. Conservation of Enok/MOZ roles in cell cycle regulation and progenitor proliferation

Several studies showed that MOZ is required to regulate cell cycle progression, notably for the G1/S transition (Sheikh et al. 2017; Flor M. Perez-Campo et al. 2014) in hematopoietic and neural stem cells, and in particular to maintain them in a quiescent state. Furthermore, MOZ catalytic activity has been shown to be required for the proliferation of hematopoietic progenitors (F. M. Perez-Campo et al. 2009), but not for their ability to generate all blood cell types. These molecular functions are conserved in Enok, as it is required to control the G1/S transition by controlling the PCNA unloading properties of Elg1 in S2 cells (Huang et al. 2016), and for the proliferation of neuroblasts during mushroom bodies development (Scott, Lee, et Luo 2001). However, we don't know whether Enok is also required for cell cycle control and progenitor proliferation during hematopoiesis.

I showed during my PhD training that the catalytic activity of Enok is not required for crystal cell differentiation; however, we did not assess the effect of the catalytic loss of function of Enok on the proliferative capacities of plasmatocytes, are responsible for the expansion of the circulating pool of hemocytes (Makhijani et al. 2011; Leitão et Sucena 2015).

Although we did not observe any reduction in circulating hemocyte number in *enok* mutants, it would be necessary to study properly plasmacyte proliferative abilities in an *enok*^{KAT} mutant context by using specific markers, such as phospho-Histone H3. Finally, it may be also interesting to analyze the effect of *enok*^{KAT} mutation on progenitors of the lymph gland in the medullary zone and the posterior lobes.

3. Mechanistic hypotheses accounting for catalytic-independent regulation of *lz* expression by Enok in larval Hematopoietic Pockets

A recent study showed that during development, Enok and its partner Br140, along with Polycomb complexes, are detected on bivalent promoters of developmentally regulated genes (Kang et al. 2017). Bivalency is achieved on promoters with specific histone marks: the first one is trimethylation of histone H3 on lysine 27 (H3K27me3), which promotes transcriptional repression, and the second is trimethylation of histone H3 on lysine 4 (H3K4me3), which promotes transcriptional activation. The authors propose a model where, in response to developmental cues, this bivalency is resolved, and the corresponding gene is either transcribed or repressed. My work demonstrated that both Enok and Br140 participate in the same process that is the control of *lz* expression during crystal cell differentiation. In addition, I showed that Enok is able to directly bind the *lz* gene in a region that is essential for its expression in circulating crystal cell precursors. Interestingly, in the *Drosophila* S2 cell line, data from MODencode project reveal that the *lz* locus lies in a H3K27me3 chromatin domain (Kharchenko et al. 2011) and a previous study by Kang *et al* show that it is heavily bound by Polycomb. According to the model proposed by Kang *et al*, it is then possible that, in response to Notch activation in plasmacytes, Enok/Br140 would allow the recruitment of activating factors triggering a switch in chromatin state at the *lz* locus from a repressed to an active state, thereby promoting *lz* expression.

In addition to this possible scenario, some evidences in the literature led me to build another hypothesis explaining the Enok-dependent *lz* regulation. It has been shown in human intestinal cells that the polyadenylation factor Symplekin is required for the direct

transcriptional inhibition of RUNX1 thus preventing intestinal goblet cell differentiation (Buchert et al. 2009). In addition, Symplekin is directly recruited on *HOXA9* promoter in KG1 AMLO immature hematopoietic cells to prevent recruitment of MOZ and MLL, therefore regulating *HOXA9* expression (Largeot et al. 2013). These mechanisms might be conserved on *Iz* regulatory regions in *Drosophila*, which possesses a Symplekin homolog. It is therefore possible that the mechanism of *Iz* regulation relies on a binding competition between Symplekin and Enok for the regulatory regions of *Iz*.

Finally, a third mechanistic hypothesis can be elaborated based on the observation that, in hematopoietic derived M1 and Jurkat T cells, MOZ has been shown to potentiate RUNX1-mediated transcription through a direct interaction (Kitabayashi 2001; Bristow 2003). This interaction is mediated by MOZ N-terminal and C-terminal parts, and has been demonstrated to be independent of MOZ catalytic activity (Kitabayashi 2001). Thus, conservation of this catalytic-independent MOZ/RUNX1 interaction across evolution would provide a simple mechanistic explanation for the non-catalytic mode of action of Enok in the control of *Iz* expression during plasmacyte to crystal cell transdifferentiation. Although the *enok*^{M31.1} mutant allele revealed that the C-terminus of Enok is not required for larval crystal cell formation in the hematopoietic pockets, it is still possible that its conserved N-terminal part might be involved in an interaction with *Iz* itself. Interestingly, while the *cis*-elements required to ensure *Iz* autoregulation during embryogenesis are located in the *Iz*-upstream region (Ferjoux et al. 2007), I identified RUNX binding sites in the *Iz*^{VT059215} region, raising the possibility that this region might also be the target of an autoregulatory loop. An interaction between Enok and *Iz* would therefore explain how Enok controls *Iz* expression *via* the third intron enhancer. This hypothesis implies that Enok and *Iz* have the capacity to interact physically, which can be tested in co-immunoprecipitation experiments. Should this hypothesis be proven, subsequent CHIP-on-CHIP experiments could be used to assess if this interaction occurs on the third intron enhancer. In addition, mutagenesis of the RUNX binding site in the *Iz*^{VT059215}-RedStinger reporter and/or endogenous *Iz* intron could provide additional elements allowing us to rule out / to establish a possible role for a conserved MOZ/RUNX interaction during Enok-dependent control of *Iz* expression in crystal cell precursors.

4. Mechanistic hypothesis accounting for Enok-dependent negative regulation of Srp/Lz-transactivation in cultured cells

It has been reported previously that, in *Drosophila* cultured Kc167 cells, Enok acts as a negative regulator of Srp/Lz-mediated transcription (Gobert et al. 2010). This repressor activity might also find its source in an interaction between Enok and Lz, which would antagonize Srp/Lz-dependent transcription. Indeed Srp and Enok could compete for interaction with Lz; alternatively, the formation of a ternary Srp/Lz/Enok complex might induce conformational changes that render the complex less efficient to promote transcription of the PPO1 and PPO2 target genes.

5. A possible conservation of KAT independent functions in the MYST family

The novel idea that there are catalytic independent functions of Enok and seemingly MOZ during development raises an interrogation: is this differential requirement for catalytic and non-catalytic functions specific of Enok/MOZ ? The high level of conservation of the MYST domains allows us to introduce the same mutation that I used to abrogate the catalytic activity of Enok in other *Drosophila* MYST proteins *in vivo*. Systematic comparison of the phenotypes induced by complete loss of function and specific catalytic abrogation should allow us to establish whether non-catalytic functions are a common feature of all *Drosophila* MYST proteins or not. Given the versatility of CRISPR/Cas9 techniques, similar approaches could be conducted in vertebrate models like Zebrafish, in order to establish whether all MYSTs lysine acetyltransferases display this dual mode of action.

D. *Drosophila* as a model to study MOZ-related diseases

It is well established that in mammals MOZ is a crucial player during hematopoietic development and this work demonstrated that Enok also plays an essential role during

Drosophila hematopoiesis. Since fusion proteins involving MOZ are associated with malignant hematopoiesis and *Drosophila* has been largely used to model human diseases, it would be interesting to express human leukemogenic MOZ versions (MOZ-CBP, MOZ-p300, MOZ-TIF2 and/or MOZ-NCoA3) in *Drosophila* hematopoietic compartments, in order to decipher the molecular mechanisms at work during those leukemic transformations.

VII. References

- Adya, N, L.H Castilla, et P.P Liu. 2000. « Function of CBF β /Bro Proteins ». *Seminars in Cell & Developmental Biology* 11 (5): 361-68. <https://doi.org/10.1006/scdb.2000.0189>.
- Akunuru, Shailaja, et Hartmut Geiger. 2016. « Aging, Clonality, and Rejuvenation of Hematopoietic Stem Cells ». *Trends in Molecular Medicine* 22 (8): 701-12. <https://doi.org/10.1016/j.molmed.2016.06.003>.
- Ali, Muzaffar, Kezhi Yan, Marie-Eve Lalonde, Cindy Degerny, Scott B. Rothbart, Brian D. Strahl, Jacques Côté, Xiang-Jiao Yang, et Tatiana G. Kutateladze. 2012. « Tandem PHD Fingers of MORF/MOZ Acetyltransferases Display Selectivity for Acetylated Histone H3 and Are Required for the Association with Chromatin ». *Journal of Molecular Biology* 424 (5): 328-38. <https://doi.org/10.1016/j.jmb.2012.10.004>.
- Anderl, Ines, Laura Vesala, Teemu O. Ihalainen, Leena-Maija Vanha-aho, István Andó, Mika Rämét, et Dan Hultmark. 2016. « Transdifferentiation and Proliferation in Two Distinct Hemocyte Lineages in *Drosophila Melanogaster* Larvae after Wasp Infection ». Édité par David S. Schneider. *PLOS Pathogens* 12 (7): e1005746. <https://doi.org/10.1371/journal.ppat.1005746>.
- Anderson, RC. 1945. « A STUDY OF THE FACTORS AFFECTING FERTILITY OF LOZENGE FEMALES OF *DROSOPHILA MELANOGASTER* ». *Genetics*.
- Appel, Elena, Sarit Weissmann, Yehuda Salzberg, Kira Orlovsky, Varda Negreanu, Michael Tsoory, Calanit Raanan, et al. 2016. « An Ensemble of Regulatory Elements Controls *Runx3* Spatiotemporal Expression in Subsets of Dorsal Root Ganglia Proprioceptive Neurons ». *Genes & Development* 30 (23): 2607-22. <https://doi.org/10.1101/gad.291484.116>.
- Aronson, B D, A L Fisher, K Blechman, M Caudy, et J P Gergen. 1997. « Groucho-Dependent and -Independent Repression Activities of Runt Domain Proteins. » *Molecular and Cellular Biology* 17 (9): 5581-87. <https://doi.org/10.1128/MCB.17.9.5581>.
- Asada, Noboru, Shoichiro Takeishi, et Paul S. Frenette. 2017. « Complexity of Bone Marrow Hematopoietic Stem Cell Niche ». *International Journal of Hematology* 106 (1): 45-54. <https://doi.org/10.1007/s12185-017-2262-9>.
- Avet-Rochex, Amelie, Karene Boyer, Cedric Polesello, Vanessa Gobert, Dani Osman, Fernando Roch, Benoit Auge, Jennifer Zanet, Marc Haenlin, et Lucas Waltzer. 2010. « An in Vivo RNA Interference Screen Identifies Gene Networks Controlling *Drosophila Melanogaster* Blood Cell Homeostasis. » *BMC Developmental Biology* 10 (1): 65. <https://doi.org/10.1186/1471-213X-10-65>.
- Bae, S. C., Y. Yamaguchi-Iwai, E. Ogawa, M. Maruyama, M. Inuzuka, H. Kagoshima, K. Shigesada, M. Satake, et Y. Ito. 1993. « Isolation of PEBP2 Alpha B CDNA Representing the Mouse Homolog of Human Acute Myeloid Leukemia Gene, AML1. » *Oncogene* 8 (3): 809-14.
- Bae, Suk-Chul, et Joong-Kook Choi. 2004. « Tumor Suppressor Activity of RUNX3 ». *Oncogene* 23 (24): 4336-40. <https://doi.org/10.1038/sj.onc.1207286>.
- Baell, Jonathan B., David J. Leaver, Stefan J. Hermans, Gemma L. Kelly, Margs S. Brennan, Natalie L. Downer, Nghi Nguyen, et al. 2018. « Inhibitors of Histone Acetyltransferases KAT6A/B Induce Senescence and Arrest Tumour Growth ». *Nature* 560 (7717): 253-57. <https://doi.org/10.1038/s41586-018-0387-5>.

- Banerjee, Utpal, Juliet R. Girard, Lauren M. Goins, et Carrie M. Spratford. 2019. « *Drosophila* as a Genetic Model for Hematopoiesis ». *Genetics* 211 (2): 367-417. <https://doi.org/10.1534/genetics.118.300223>.
- Baron, Margaret H. 2013. « Concise Review: Early Embryonic Erythropoiesis: Not so Primitive After All ». *STEM CELLS* 31 (5): 849-56. <https://doi.org/10.1002/stem.1342>.
- Bataillé, Laetitia, Benoit Augé, Géraldine Ferjoux, Marc Haenlin, et Lucas Waltzer. 2005. « Resolving Embryonic Blood Cell Fate Choice in *Drosophila*: Interplay of GCM and RUNX Factors », 11.
- Benmimoun, Billel, Cédric Polesello, Marc Haenlin, et Lucas Waltzer. 2015. « The EBF Transcription Factor Collier Directly Promotes *Drosophila* Blood Cell Progenitor Maintenance Independently of the Niche ». *Proceedings of the National Academy of Sciences* 112 (29): 9052-57. <https://doi.org/10.1073/pnas.1423967112>.
- Bertrand, J. Y. 2005. « Three Pathways to Mature Macrophages in the Early Mouse Yolk Sac ». *Blood* 106 (9): 3004-11. <https://doi.org/10.1182/blood-2005-02-0461>.
- Bertrand, J. Y., J. L. Cisson, D. L. Stachura, et D. Traver. 2010. « Notch Signaling Distinguishes 2 Waves of Definitive Hematopoiesis in the Zebrafish Embryo ». *Blood* 115 (14): 2777-83. <https://doi.org/10.1182/blood-2009-09-244590>.
- Bethlenfalvai, N.C., et M. Block. 1970. « Fetal Erythropoiesis ». *Acta Haematologica* 44 (4): 240-45. <https://doi.org/10.1159/000208685>.
- Bidla, G., M. S. Dushay, et U. Theopold. 2007. « Crystal Cell Rupture after Injury in *Drosophila* Requires the JNK Pathway, Small GTPases and the TNF Homolog Eiger ». *Journal of Cell Science* 120 (7): 1209-15. <https://doi.org/10.1242/jcs.03420>.
- Binggeli, Olivier, Claudine Neyen, Mickael Poidevin, et Bruno Lemaitre. 2014. « Prophenoloxidase Activation Is Required for Survival to Microbial Infections in *Drosophila* ». Édité par David S. Schneider. *PLoS Pathogens* 10 (5): e1004067. <https://doi.org/10.1371/journal.ppat.1004067>.
- Borrow, Julian, Vincent P. Stanton, J. Michael Andresen, Reinhard Becher, Frederick G. Behm, Raju S. K. Chaganti, Curt I. Civin, et al. 1996. « The translocation t(8;16)(p11;p13) of acute myeloid leukaemia fuses a putative acetyltransferase to the CREB-binding protein ». *Nature Genetics* 14 (1): 33-41. <https://doi.org/10.1038/ng0996-33>.
- Bras, S., S. Martin-Lannere, V. Gobert, B. Auge, O. Breig, M. Sanial, M. Yamaguchi, M. Haenlin, A. Plessis, et L. Waltzer. 2012. « Myeloid Leukemia Factor Is a Conserved Regulator of RUNX Transcription Factor Activity Involved in Hematopoiesis ». *Proceedings of the National Academy of Sciences* 109 (13): 4986-91. <https://doi.org/10.1073/pnas.1117317109>.
- Bray, Sarah J. 2006. « Notch Signalling: A Simple Pathway Becomes Complex ». *Nature Reviews Molecular Cell Biology* 7 (9): 678-89. <https://doi.org/10.1038/nrm2009>.
- Bristow, C. A. P. 2003. « Transcriptional Regulation of the Human MIP-1 α Promoter by RUNX1 and MOZ ». *Nucleic Acids Research* 31 (11): 2735-44. <https://doi.org/10.1093/nar/gkg401>.

- Bruijn, M. F.T.R. de. 2000. « Definitive Hematopoietic Stem Cells First Develop within the Major Arterial Regions of the Mouse Embryo ». *The EMBO Journal* 19 (11): 2465-74. <https://doi.org/10.1093/emboj/19.11.2465>.
- Bunt, Stephanie, Clare Hooley, Nan Hu, Catherine Scahill, Helen Weavers, et Helen Skaer. 2010. « Hemocyte-Secreted Type IV Collagen Enhances BMP Signaling to Guide Renal Tubule Morphogenesis in *Drosophila* ». *Developmental Cell* 19 (2): 296-306. <https://doi.org/10.1016/j.devcel.2010.07.019>.
- Carapeti, Melina, Ricardo C.T Aguiar, Ann E Watmore, John M Goldman, et Nicholas C.P Cross. 1999. « Consistent Fusion of MOZ and TIF2 in AML with Inv(8)(P11q13) ». *Cancer Genetics and Cytogenetics* 113 (1): 70-72. [https://doi.org/10.1016/S0165-4608\(99\)00007-2](https://doi.org/10.1016/S0165-4608(99)00007-2).
- Cevik, Duygu, Meryl Acker, Camilla Michalski, et J. Roger Jacobs. 2019. « Pericardin, a *Drosophila* Collagen, Facilitates Accumulation of Hemocytes at the Heart ». *Developmental Biology* 454 (1): 52-65. <https://doi.org/10.1016/j.ydbio.2019.06.006>.
- Chaffanet, Max, Laetitia Gressin, Claude Preudhomme, Valérie Soenen-Cornu, Daniel Birnbaum, et Marie-Josèphe Pébusque. 2000. « MOZ Is Fused to P300 in an Acute Monocytic Leukemia with t(8;22) », 7.
- Champagne, Karen S., Nehmé Saksouk, Pedro V. Peña, Kyle Johnson, Mukta Ullah, Xiang-Jiao Yang, Jacques Côté, et Tatiana G. Kutateladze. 2008. « The Crystal Structure of the ING5 PHD Finger in Complex with an H3K4me3 Histone Peptide ». *Proteins: Structure, Function, and Bioinformatics* 72 (4): 1371-76. <https://doi.org/10.1002/prot.22140>.
- Champagne, Nathalie, Nadine Pelletier, et Xiang-Jiao Yang. 2001. « The Monocytic Leukemia Zinc Finger Protein MOZ Is a Histone Acetyltransferase », 6.
- Chan, Edward M., Rebecca J. Chan, Elisha M. Comer, Robert J. Goulet, Colin D. Crean, Zachary D. Brown, Amy M. Fruehwald, et al. 2007. « MOZ and MOZ-CBP Cooperate with NF-KB to Activate Transcription from NF-KB-Dependent Promoters ». *Experimental Hematology* 35 (12): 1782-92. <https://doi.org/10.1016/j.exphem.2007.07.015>.
- Chang, A. N., A. B. Cantor, Y. Fujiwara, M. B. Lodish, S. Droho, J. D. Crispino, et S. H. Orkin. 2002. « GATA-Factor Dependence of the Multitype Zinc-Finger Protein FOG-1 for Its Essential Role in Megakaryopoiesis ». *Proceedings of the National Academy of Sciences* 99 (14): 9237-42. <https://doi.org/10.1073/pnas.142302099>.
- Charroux, B., et J. Royet. 2009. « Elimination of Plasmatocytes by Targeted Apoptosis Reveals Their Role in Multiple Aspects of the *Drosophila* Immune Response ». *Proceedings of the National Academy of Sciences* 106 (24): 9797-9802. <https://doi.org/10.1073/pnas.0903971106>.
- Chen, Michael J., Tomomasa Yokomizo, Brandon M. Zeigler, Elaine Dzierzak, et Nancy A. Speck. 2009. « Runx1 Is Required for the Endothelial to Haematopoietic Cell Transition but Not Thereafter ». *Nature* 457 (7231): 887-91. <https://doi.org/10.1038/nature07619>.
- Chi, Yali, Zhibin Huang, Qi Chen, Xiaojie Xiong, Kemin Chen, Jin Xu, Yiyue Zhang, et Wenqing Zhang. 2018. « Loss of *Runx1* Function Results in B Cell Immunodeficiency but Not T Cell in Adult Zebrafish ». *Open Biology* 8 (7): 180043. <https://doi.org/10.1098/rsob.180043>.

- Chuang, Linda Shyue Huey, Kosei Ito, et Yoshiaki Ito. 2013. « RUNX Family: Regulation and Diversification of Roles through Interacting Proteins ». *International Journal of Cancer* 132 (6): 1260-71. <https://doi.org/10.1002/ijc.27964>.
- Cieślak-Pobuda, Artur, Viktoria Knoflach, Mikael V. Ringh, Joachim Stark, Wirginia Likus, Krzysztof Siemianowicz, Saeid Ghavami, Andrzej Hudecki, Jason L. Green, et Marek J. Łos. 2017. « Transdifferentiation and Reprogramming: Overview of the Processes, Their Similarities and Differences ». *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* 1864 (7): 1359-69. <https://doi.org/10.1016/j.bbamcr.2017.04.017>.
- Clarke, Raedun L., Amanda D. Yzaguirre, Yumi Yashiro-Ohtani, Antoine Bondue, Cedric Blanpain, Warren S. Pear, Nancy A. Speck, et Gordon Keller. 2013. « The expression of Sox17 identifies and regulates haemogenic endothelium ». *Nature Cell Biology* 15 (avril): 502.
- Crowley, J A, Y Wang, A P Rapoport, et Y Ning. 2005. « Detection of MOZ-CBP Fusion in Acute Myeloid Leukemia with 8;16 Translocation ». *Leukemia* 19 (12): 2344-45. <https://doi.org/10.1038/sj.leu.2403971>.
- Crozatier, Michèle, Jean-Michel Ubeda, Alain Vincent, et Marie Meister. 2004. « Cellular Immune Response to Parasitization in *Drosophila* Requires the EBF Orthologue Collier ». Édité par Michael Levine. *PLoS Biology* 2 (8): e196. <https://doi.org/10.1371/journal.pbio.0020196>.
- Daga, A., C. A. Karlovich, K. Dumstrei, et U. Banerjee. 1996. « Patterning of Cells in the *Drosophila* Eye by Lozenge, Which Shares Homologous Domains with AML1. » *Genes & Development* 10 (10): 1194-1205. <https://doi.org/10.1101/gad.10.10.1194>.
- De Kouchkovsky, I, et M Abdul-Hay. 2016. « 'Acute Myeloid Leukemia: A Comprehensive Review and 2016 Update' ». *Blood Cancer Journal* 6 (7): e441-e441. <https://doi.org/10.1038/bcj.2016.50>.
- Defaye, Arnaud, Iwan Evans, Michèle Crozatier, Will Wood, Bruno Lemaitre, et François Leulier. 2009. « Genetic Ablation of *Drosophila* Phagocytes Reveals Their Contribution to Both Development and Resistance to Bacterial Infection ». *Journal of Innate Immunity* 1 (4): 322-34. <https://doi.org/10.1159/000210264>.
- Deguchi, Kenji, Paul M Ayton, Melina Carapeti, Jeffery L Kutok, Cynthia S Snyder, Ifor R Williams, Nicholas C.P Cross, Christopher K Glass, Michael L Cleary, et D.Gary Gilliland. 2003. « MOZ-TIF2-Induced Acute Myeloid Leukemia Requires the MOZ Nucleosome Binding Motif and TIF2-Mediated Recruitment of CBP ». *Cancer Cell* 3 (3): 259-71. [https://doi.org/10.1016/S1535-6108\(03\)00051-5](https://doi.org/10.1016/S1535-6108(03)00051-5).
- Demarest, Stephen J., Maria Martinez-Yamout, John Chung, Hongwu Chen, Wei Xu, H. Jane Dyson, Ronald M. Evans, et Peter E. Wright. 2002. « Mutual Synergistic Folding in Recruitment of CBP/P300 by P160 Nuclear Receptor Coactivators ». *Nature* 415 (6871): 549-53. <https://doi.org/10.1038/415549a>.
- Djiane, Alexandre, Sophie Zaessinger, A. Burcu Babaoğlan, et Sarah J. Bray. 2014. « Notch Inhibits Yorkie Activity in *Drosophila* Wing Discs ». Édité par Franck Pichaud. *PLoS ONE* 9 (8): e106211. <https://doi.org/10.1371/journal.pone.0106211>.

- Draper, Julia E., Patrycja Sroczyńska, Olga Tsoulaki, Hui Sun Leong, Muhammad Z. H. Fadlullah, Crispin Miller, Valerie Kouskoff, et Georges Lacaud. 2016. « RUNX1B Expression Is Highly Heterogeneous and Distinguishes Megakaryocytic and Erythroid Lineage Fate in Adult Mouse Hematopoiesis ». Édité par H. Leighton Grimes. *PLOS Genetics* 12 (1): e1005814. <https://doi.org/10.1371/journal.pgen.1005814>.
- Dreveny, I., S. E. Deeves, J. Fulton, B. Yue, M. Messmer, A. Bhattacharya, H. M. Collins, et D. M. Heery. 2014. « The Double PHD Finger Domain of MOZ/MYST3 Induces -Helical Structure of the Histone H3 Tail to Facilitate Acetylation and Methylation Sampling and Modification ». *Nucleic Acids Research* 42 (2): 822-35. <https://doi.org/10.1093/nar/gkt931>.
- Dudzic, Jan P., Shu Kondo, Ryu Ueda, Casey M. Bergman, et Bruno Lemaitre. 2015. « *Drosophila* Innate Immunity: Regional and Functional Specialization of Prophenoloxidasés ». *BMC Biology* 13 (1): 81. <https://doi.org/10.1186/s12915-015-0193-6>.
- Dulak, Austin M, Petar Stojanov, Shouyong Peng, Michael S Lawrence, Cameron Fox, Chip Stewart, Santhoshi Bandla, et al. 2013. « Exome and Whole-Genome Sequencing of Esophageal Adenocarcinoma Identifies Recurrent Driver Events and Mutational Complexity ». *Nature Genetics* 45 (5): 478-86. <https://doi.org/10.1038/ng.2591>.
- Duvic, Bernard, Jules A Hoffmann, Marie Meister, et Julien Royet. 2002. « Notch Signaling Controls Lineage Specification during *Drosophila* Larval Hematopoiesis ». *Current Biology* 12 (22): 1923-27. [https://doi.org/10.1016/S0960-9822\(02\)01297-6](https://doi.org/10.1016/S0960-9822(02)01297-6).
- Dyer, Jamie O., Arnob Dutta, Madelaine Gogol, Vikki M. Weake, George Dialynas, Xilan Wu, Christopher Seidel, et al. 2017. « Myeloid Leukemia Factor Acts in a Chaperone Complex to Regulate Transcription Factor Stability and Gene Expression ». *Journal of Molecular Biology* 429 (13): 2093-2107. <https://doi.org/10.1016/j.jmb.2016.10.026>.
- Elagib, K. E. 2003. « RUNX1 and GATA-1 Coexpression and Cooperation in Megakaryocytic Differentiation ». *Blood* 101 (11): 4333-41. <https://doi.org/10.1182/blood-2002-09-2708>.
- Erickson, P, et J Gao. 1992. « Identification of Breakpoints in t (8;21) Acute Myelogenous Leukemia and Isolation of a Fusion Transcript, AML1 IETO, With Similarity to *Drosophila* Segmentation Gene, Runt », 8.
- Ferguson, Gabriel B., et Julian A. Martinez-Agosto. 2017. « The TEAD Family Transcription Factor Scalloped Regulates Blood Progenitor Maintenance and Proliferation in *Drosophila* through PDGF/VEGFR Receptor (Pvr) Signaling ». *Developmental Biology* 425 (1): 21-32. <https://doi.org/10.1016/j.ydbio.2017.03.016>.
- Ferguson, Gabriel B., et Julian A. Martinez-Agosto. 2014. « Yorkie and Scalloped Signaling Regulates Notch-Dependent Lineage Specification during *Drosophila* Hematopoiesis ». *Current Biology* 24 (22): 2665-72. <https://doi.org/10.1016/j.cub.2014.09.081>.
- Ferjoux, Géraldine, Benoit Augé, Karène Boyer, Marc Haenlin, et Lucas Waltzer. 2007. « A GATA/RUNX Cis-Regulatory Module Couples *Drosophila* Blood Cell Commitment and Differentiation into Crystal Cells ». *Developmental Biology* 305 (2): 726-34. <https://doi.org/10.1016/j.ydbio.2007.03.010>.

- Fossett, N., K. Hyman, K. Gajewski, S. H. Orkin, et R. A. Schulz. 2003. « Combinatorial Interactions of Serpent, Lozenge, and U-Shaped Regulate Crystal Cell Lineage Commitment during *Drosophila* Hematopoiesis ». *Proceedings of the National Academy of Sciences* 100 (20): 11451-56. <https://doi.org/10.1073/pnas.1635050100>.
- Frame, Jenna M., Katherine H. Fegan, Simon J. Conway, Kathleen E. McGrath, et James Palis. 2016. « Definitive Hematopoiesis in the Yolk Sac Emerges from Wnt-Responsive Hemogenic Endothelium Independently of Circulation and Arterial Identity: EMPs Arise from Arteriovenous Hemogenic Endothelia ». *STEM CELLS* 34 (2): 431-44. <https://doi.org/10.1002/stem.2213>.
- Frame, Jenna M., Kathleen E. McGrath, et James Palis. 2013. « Erythro-Myeloid Progenitors: “Definitive” Hematopoiesis in the Conceptus Prior to the Emergence of Hematopoietic Stem Cells ». *Blood Cells, Molecules, and Diseases* 51 (4): 220-25. <https://doi.org/10.1016/j.bcmd.2013.09.006>.
- Fujiwara, Y., C. P. Browne, K. Cunniff, S. C. Goff, et S. H. Orkin. 1996. « Arrested Development of Embryonic Red Cell Precursors in Mouse Embryos Lacking Transcription Factor GATA-1. » *Proceedings of the National Academy of Sciences* 93 (22): 12355-58. <https://doi.org/10.1073/pnas.93.22.12355>.
- Gaidzik, Verena I., Lars Bullinger, Richard F. Schlenk, Andreas S. Zimmermann, Jürgen Röck, Peter Paschka, Andrea Corbacioglu, et al. 2011. « *RUNX1* Mutations in Acute Myeloid Leukemia: Results From a Comprehensive Genetic and Clinical Analysis From the AML Study Group ». *Journal of Clinical Oncology* 29 (10): 1364-72. <https://doi.org/10.1200/JCO.2010.30.7926>.
- Gekas, Christos, Françoise Dieterlen-Lièvre, Stuart H. Orkin, et Hanna K.A. Mikkola. 2005. « The Placenta Is a Niche for Hematopoietic Stem Cells ». *Developmental Cell* 8 (3): 365-75. <https://doi.org/10.1016/j.devcel.2004.12.016>.
- Gergen, J.Peter, et Eric Wieschaus. 1986. « Dosage requirements for runt in the segmentation of *Drosophila* embryos ». *Cell* 45 (2): 289-99. [https://doi.org/10.1016/0092-8674\(86\)90393-4](https://doi.org/10.1016/0092-8674(86)90393-4).
- Ghosh, S, S Mandal, et L Mandal. 2018. « Detecting proliferation of adult hemocytes in *Drosophila* by BrdU incorporation and PH3 expression in response to bacterial infection ». *Wellcome Open Res* 2018, 3:47, 2018. (<https://doi.org/10.12688/wellcomeopenres.14560.2>).
- Ghosh, Saikat, Arashdeep Singh, Sudip Mandal, et Lolitika Mandal. 2015. « Active Hematopoietic Hubs in *Drosophila* Adults Generate Hemocytes and Contribute to Immune Response ». *Developmental Cell* 33 (4): 478-88. <https://doi.org/10.1016/j.devcel.2015.03.014>.
- Gobert, Vanessa, Marc Haenlin, et Lucas Waltzer. 2012. « Myeloid Leukemia Factor: A Return Ticket from Human Leukemia to Fly Hematopoiesis ». *Transcription* 3 (5): 250-54. <https://doi.org/10.4161/trns.21490>.
- Gobert, Vanessa, Dani Osman, Stéphanie Bras, Benoit Augé, Muriel Boube, Henri-Marc Bourbon, Thomas Horn, Michael Boutros, Marc Haenlin, et Lucas Waltzer. 2010a. « A Genome-Wide RNA Interference Screen Identifies a Differential Role of the Mediator CDK8 Module Subunits for GATA/ *RUNX*-Activated Transcription in *Drosophila* ».

- Molecular and Cellular Biology* 30 (11): 2837-48. <https://doi.org/10.1128/MCB.01625-09>.
- Gold, Katrina S., et Katja Brückner. 2015. « Macrophages and Cellular Immunity in *Drosophila Melanogaster* ». *Seminars in Immunology* 27 (6): 357-68. <https://doi.org/10.1016/j.smim.2016.03.010>.
- Goldfarb, Adam N. 2009. « Megakaryocytic Programming by a Transcriptional Regulatory Loop: A Circle Connecting RUNX1, GATA-1, and P-TEFb ». *Journal of Cellular Biochemistry* 107 (3): 377-82. <https://doi.org/10.1002/jcb.22142>.
- Goll, M. G. 2002. « Histone Modification and Replacement in Chromatin Activation ». *Genes & Development* 16 (14): 1739-42. <https://doi.org/10.1101/gad.1013902>.
- Gomez Perdiguero, Elisa, Kay Klapproth, Christian Schulz, Katrin Busch, Emanuele Azzoni, Lucile Crozet, Hannah Garner, et al. 2015. « Tissue-Resident Macrophages Originate from Yolk-Sac-Derived Erythro-Myeloid Progenitors ». *Nature* 518 (7540): 547-51. <https://doi.org/10.1038/nature13989>.
- Grant, Patrick A. 2001. « A Tale of Histone Modifications », 6.
- Graves, Hillary K., Sarah E. Woodfield, Chih-Chao Yang, Georg Halder, et Andreas Bergmann. 2012. « Notch Signaling Activates Yorkie Non-Cell Autonomously in *Drosophila* ». Édité par Madhuri Kango-Singh. *PLoS ONE* 7 (6): e37615. <https://doi.org/10.1371/journal.pone.0037615>.
- Grimwade, D., R. K. Hills, A. V. Moorman, H. Walker, S. Chatters, A. H. Goldstone, K. Wheatley, C. J. Harrison, A. K. Burnett, et on behalf of the National Cancer Research Institute Adult Leukaemia Working Group. 2010. « Refinement of Cytogenetic Classification in Acute Myeloid Leukemia: Determination of Prognostic Significance of Rare Recurring Chromosomal Abnormalities among 5876 Younger Adult Patients Treated in the United Kingdom Medical Research Council Trials ». *Blood* 116 (3): 354-65. <https://doi.org/10.1182/blood-2009-11-254441>.
- Grisolano, J. L., J. O'Neal, J. Cain, et M. H. Tomasson. 2003. « An Activated Receptor Tyrosine Kinase, TEL/PDGF R, Cooperates with AML1/ETO to Induce Acute Myeloid Leukemia in Mice ». *Proceedings of the National Academy of Sciences* 100 (16): 9506-11. <https://doi.org/10.1073/pnas.1531730100>.
- Hadland, B. K. 2004. « A Requirement for Notch1 Distinguishes 2 Phases of Definitive Hematopoiesis during Development ». *Blood* 104 (10): 3097-3105. <https://doi.org/10.1182/blood-2004-03-1224>.
- Haferlach, Claudia, Niroshan Nadarajah, Wolfgang Kern, Susanne Schnittger, et Torsten Haferlach. 2014. « The *RUNX1* Gene Is Altered in 26% of AML Patients Either By Translocation, Mutation, Gain or Deletion ». *Blood* 124 (21): 123.
- Harikumar, Arigela, et Eran Meshorer. 2015. « Chromatin Remodeling and Bivalent Histone Modifications in Embryonic Stem Cells ». *EMBO Reports* 16 (12): 1609-19. <https://doi.org/10.15252/embr.201541011>.
- Hashimoto, Daigo, Andrew Chow, Clara Noizat, Pearline Teo, Mary Beth Beasley, Marylene Leboeuf, Christian D. Becker, et al. 2013. « Tissue-Resident Macrophages Self-Maintain

- Locally throughout Adult Life with Minimal Contribution from Circulating Monocytes ». *Immunity* 38 (4): 792-804. <https://doi.org/10.1016/j.immuni.2013.04.004>.
- Hibiya, Kenta, Takuo Katsumoto, Takashi Kondo, Issay Kitabayashi, et Akira Kudo. 2009. « Brpf1, a Subunit of the MOZ Histone Acetyl Transferase Complex, Maintains Expression of Anterior and Posterior Hox Genes for Proper Patterning of Craniofacial and Caudal Skeletons ». *Developmental Biology* 329 (2): 176-90. <https://doi.org/10.1016/j.ydbio.2009.02.021>.
- Ho, I-Cheng, Tzong-Shyuan Tai, et Sung-Yun Pai. 2009. « GATA3 and the T-Cell Lineage: Essential Functions before and after T-Helper-2-Cell Differentiation ». *Nature Reviews Immunology* 9 (2): 125-35. <https://doi.org/10.1038/nri2476>.
- Holbert, Marc A., Timothy Sikorski, Juliana Carten, Danielle Snowflack, Santosh Hodawadkar, et Ronen Marmorstein. 2007. « The Human Monocytic Leukemia Zinc Finger Histone Acetyltransferase Domain Contains DNA-Binding Activity Implicated in Chromatin Targeting ». *Journal of Biological Chemistry* 282 (50): 36603-13. <https://doi.org/10.1074/jbc.M705812200>.
- Holz, A. 2003. « The Two Origins of Hemocytes in *Drosophila* ». *Development* 130 (20): 4955-62. <https://doi.org/10.1242/dev.00702>.
- Honti, Viktor, Éva Kurucz, Gábor Csordás, Barbara Laurinyecz, Róbert Márkus, et István Andó. 2009. « In Vivo Detection of Lamellocytes in *Drosophila Melanogaster* ». *Immunology Letters* 126 (1-2): 83-84. <https://doi.org/10.1016/j.imlet.2009.08.004>.
- Huang, Fu, Anita Saraf, Laurence Florens, Thomas Kusch, Selene K. Swanson, Leanne T. Szerszen, Ge Li, et al. 2016. « The Enok Acetyltransferase Complex Interacts with Elg1 and Negatively Regulates PCNA Unloading to Promote the G1/S Transition ». *Genes & Development*, mai, genesdev;gad.271429.115v1. <https://doi.org/10.1101/gad.271429.115>.
- Huber, Tara L., Valerie Kouskoff, H. Joerg Fehling, James Palis, et Gordon Keller. 2004. « Haemangioblast commitment is initiated in the primitive streak of the mouse embryo ». *Nature* 432 (7017): 625-30. <https://doi.org/10.1038/nature03122>.
- Irving, Phil, Jean-Michel Ubeda, Daniel Doucet, Laurent Troxler, Marie Lagueux, Daniel Zachary, Jules A. Hoffmann, Charles Hetru, et Marie Meister. 2005. « New Insights into *Drosophila* Larval Haemocyte Functions through Genome-Wide Analysis ». *Cellular Microbiology* 7 (3): 335-50. <https://doi.org/10.1111/j.1462-5822.2004.00462.x>.
- Ito, Yoshiaki. 2008. « RUNX Genes in Development and Cancer: Regulation of Viral Gene Expression and the Discovery of RUNX Family Genes ». In *Advances in Cancer Research*, 99:33-76. Elsevier. [https://doi.org/10.1016/S0065-230X\(07\)99002-8](https://doi.org/10.1016/S0065-230X(07)99002-8).
- Ito, Yoshiaki, Suk-Chul Bae, et Linda Shyue Huey Chuang. 2015. « The RUNX Family: Developmental Regulators in Cancer ». *Nature Reviews Cancer* 15 (2): 81-95. <https://doi.org/10.1038/nrc3877>.
- Iwasaki, Hiromi, Shin-ichi Mizuno, Richard A Wells, Alan B Cantor, Sumiko Watanabe, et Koichi Akashi. 2003. « GATA-1 Converts Lymphoid and Myelomonocytic Progenitors into the Megakaryocyte/Erythrocyte Lineages ». *Immunity* 19 (3): 451-62. [https://doi.org/10.1016/S1074-7613\(03\)00242-5](https://doi.org/10.1016/S1074-7613(03)00242-5).

- Jacobson, L. O., E. L. Simmons, E. K. Marks, et J. H. Eldredge. 1951. « Recovery from Radiation Injury ». *Science* 113 (2940): 510-11. <https://doi.org/10.1126/science.113.2940.510>.
- Jiang, Ming, Ju Zhang, Lili Qian, Yuhui Miao, Weiguo Song, Hanyuan Liu, et Rui Li. 2019. « MOZ Forms an Autoregulatory Feedback Loop with MiR-223 in AML and Monocyte/Macrophage Development ». *iScience* 11 (janvier): 189-204. <https://doi.org/10.1016/j.isci.2018.12.016>.
- Jopling, Chris, Stephanie Boue, et Juan Carlos Izpisua Belmonte. 2011. « Dedifferentiation, Transdifferentiation and Reprogramming: Three Routes to Regeneration ». *Nature Reviews Molecular Cell Biology* 12 (2): 79-89. <https://doi.org/10.1038/nrm3043>.
- Kamachi, Yusuke, Eiko Ogawa, Maki Asano, Seiko Ishida, Yota Murakami, Masanobu Satake, Yoshiaki Ito, et KATSUYA SHIGESADAI. 1990. « Purification of a Mouse Nuclear Factor That Binds to Both the A and B Cores of the Polyomavirus Enhancer » 64: 12.
- Kaminker, J S, S Rajan, T. Lebestky, H Yan, et U Banerjee. 2001. « Redundant Function of Big Brother and Brother ». *Development*, 10.
- Kang, Hyuckjoon, Youngsook L. Jung, Kyle A. McElroy, Barry M. Zee, Heather A. Wallace, Jessica L. Woolnough, Peter J. Park, et Mitzi I. Kuroda. 2017. « Bivalent Complexes of PRC1 with Orthologs of BRD4 and MOZ/MORF Target Developmental Genes in *Drosophila* ». *Genes & Development* 31 (19): 1988-2002. <https://doi.org/10.1101/gad.305987.117>.
- Katsumoto, T. 2006. « MOZ Is Essential for Maintenance of Hematopoietic Stem Cells ». *Genes & Development* 20 (10): 1321-30. <https://doi.org/10.1101/gad.1393106>.
- Kelly, Louise M., et D. Gary Gilliland. 2002. « GENETICS OF MYELOID LEUKEMIAS ». *Annual Review of Genomics and Human Genetics* 3 (1): 179-98. <https://doi.org/10.1146/annurev.genom.3.032802.115046>.
- Kieusseian, A., P. B. de la Grange, O. Burlen-Defranoux, I. Godin, et A. Cumano. 2012. « Immature Hematopoietic Stem Cells Undergo Maturation in the Fetal Liver ». *Development* 139 (19): 3521-30. <https://doi.org/10.1242/dev.079210>.
- Kindle, K. B., P. J. F. Troke, H. M. Collins, S. Matsuda, D. Bossi, C. Bellodi, E. Kalkhoven, et al. 2005. « MOZ-TIF2 Inhibits Transcription by Nuclear Receptors and P53 by Impairment of CBP Function ». *Molecular and Cellular Biology* 25 (3): 988-1002. <https://doi.org/10.1128/MCB.25.3.988-1002.2005>.
- Kingsley, P. D. 2004. « Yolk Sac-Derived Primitive Erythroblasts Enucleate during Mammalian Embryogenesis ». *Blood* 104 (1): 19-25. <https://doi.org/10.1182/blood-2003-12-4162>.
- . 2006. « “Maturation” Globin Switching in Primary Primitive Erythroid Cells ». *Blood* 107 (4): 1665-72. <https://doi.org/10.1182/blood-2005-08-3097>.
- Kitabayashi, I. 2001. « Activation of AML1-Mediated Transcription by MOZ and Inhibition by the MOZ-CBP Fusion Protein ». *The EMBO Journal* 20 (24): 7184-96. <https://doi.org/10.1093/emboj/20.24.7184>.
- Kitabayashi, Issay, Kohmei Ida, Fumiko Morohoshi, Akihiko Yokoyama, Naoko Mitsuhashi, Kimiko Shimizu, Nobuo Nomura, Yasuhide Hayashi, et Misao Ohki. 1998. « The AML1-MTG8 Leukemic Fusion Protein Forms a Complex with a Novel Member of the

- MTG8(ETO/CDR) Family, MTGR1 ». *Molecular and Cellular Biology* 18 (2): 846-58. <https://doi.org/10.1128/MCB.18.2.846>.
- Kovall, Rhett A., Brian Gebelein, David Sprinzak, et Raphael Kopan. 2017. « The Canonical Notch Signaling Pathway: Structural and Biochemical Insights into Shape, Sugar, and Force ». *Developmental Cell* 41 (3): 228-41. <https://doi.org/10.1016/j.devcel.2017.04.001>.
- Krzemien, Joanna, Justine Oyallon, Michèle Crozatier, et Alain Vincent. 2010. « Hematopoietic Progenitors and Hemocyte Lineages in the *Drosophila* Lymph Gland ». *Developmental Biology* 346 (2): 310-19. <https://doi.org/10.1016/j.ydbio.2010.08.003>.
- Kubota, Takashi, Kohei Nishimura, Masato T. Kanemaki, et Anne D. Donaldson. 2013. « The Elg1 Replication Factor C-like Complex Functions in PCNA Unloading during DNA Replication ». *Molecular Cell* 50 (2): 273-80. <https://doi.org/10.1016/j.molcel.2013.02.012>.
- Kulkarni, Madhura, Tuan Zea Tan, Nurfarhanah Bte Syed Sulaiman, John M. Lamar, Prashali Bansal, Jianzhou Cui, Yiting Qiao, et Yoshiaki Ito. 2018. « RUNX1 and RUNX3 Protect against YAP-Mediated EMT, Stem-Ness and Shorter Survival Outcomes in Breast Cancer ». *Oncotarget* 9 (18). <https://doi.org/10.18632/oncotarget.24419>.
- Kumano, Keiki, Shigeru Chiba, Atsushi Kunisato, Masataka Sata, Toshiki Saito, Etsuko Nakagami-Yamaguchi, Tomoyuki Yamaguchi, et al. 2003. « Notch1 but Not Notch2 Is Essential for Generating Hematopoietic Stem Cells from Endothelial Cells ». *Immunity* 18 (5): 699-711. [https://doi.org/10.1016/S1074-7613\(03\)00117-1](https://doi.org/10.1016/S1074-7613(03)00117-1).
- Lacaud, Georges, et Valerie Kouskoff. 2017. « Hemangioblast, Hemogenic Endothelium, and Primitive versus Definitive Hematopoiesis ». *Experimental Hematology* 49 (mai): 19-24. <https://doi.org/10.1016/j.exphem.2016.12.009>.
- Lanot, René, Daniel Zachary, François Holder, et Marie Meister. 2001. « Postembryonic Hematopoiesis in *Drosophila* ». *Developmental Biology* 230 (2): 243-57. <https://doi.org/10.1006/dbio.2000.0123>.
- Largeot, Anne, Flor Maria Perez-Campo, Elli Marinopoulou, Michael Lie-a-Ling, Valerie Kouskoff, et Georges Lacaud. 2016. « Expression of the MOZ-TIF2 Oncoprotein in Mice Represses Senescence ». *Experimental Hematology* 44 (4): 231-237.e4. <https://doi.org/10.1016/j.exphem.2015.12.006>.
- Lebestky, T. 2000. « Specification of *Drosophila* Hematopoietic Lineage by Conserved Transcription Factors ». *Science* 288 (5463): 146-49. <https://doi.org/10.1126/science.288.5463.146>.
- . 2003. « A Serrate-Expressing Signaling Center Controls *Drosophila* Hematopoiesis ». *Genes & Development* 17 (3): 348-53. <https://doi.org/10.1101/gad.1052803>.
- Lee, Gap Ryol, Patrick E. Fields, et Richard A. Flavell. 2001. « Regulation of IL-4 Gene Expression by Distal Regulatory Elements and GATA-3 at the Chromatin Level ». *Immunity* 14 (4): 447-59. [https://doi.org/10.1016/S1074-7613\(01\)00125-X](https://doi.org/10.1016/S1074-7613(01)00125-X).
- Lee, Kenneth K., et Jerry L. Workman. 2007. « Histone Acetyltransferase Complexes: One Size Doesn't Fit All ». *Nature Reviews Molecular Cell Biology* 8 (4): 284-95. <https://doi.org/10.1038/nrm2145>.

- Leitão, Alexandre B, et Élio Sucena. 2015. « *Drosophila* Sessile Hemocyte Clusters Are True Hematopoietic Tissues That Regulate Larval Blood Cell Differentiation ». *ELife* 4 (février): e06166. <https://doi.org/10.7554/eLife.06166>.
- Letourneau, Manon, Francois Lapraz, Anurag Sharma, Nathalie Vanzo, Lucas Waltzer, et Michèle Crozatier. 2016. « *Drosophila* Hematopoiesis under Normal Conditions and in Response to Immune Stress ». *FEBS Letters* 590 (22): 4034-51. <https://doi.org/10.1002/1873-3468.12327>.
- Levanon, D., R. E. Goldstein, Y. Bernstein, H. Tang, D. Goldenberg, S. Stifani, Z. Paroush, et Y. Groner. 1998. « Transcriptional Repression by AML1 and LEF-1 Is Mediated by the TLE/Groucho Corepressors ». *Proceedings of the National Academy of Sciences* 95 (20): 11590-95. <https://doi.org/10.1073/pnas.95.20.11590>.
- Levanon, D., V. Negreanu, Y. Bernstein, I. Bar-Am, L. Avivi, et Y. Groner. 1994. « AML1, AML2, and AML3, the Human Members of the runt domain Gene-Family: cDNA Structure, Expression, and Chromosomal Localization ». *Genomics* 23 (2): 425-32. <https://doi.org/10.1006/geno.1994.1519>.
- Li, Lequn, Nikolaos Patsoukis, Victoria Petkova, et Vassiliki A. Boussiotis. 2012. « Runx1 and Runx3 Are Involved in the Generation and Function of Highly Suppressive IL-17-Producing T Regulatory Cells ». Édité par Ciriaco A. Piccirillo. *PLoS ONE* 7 (9): e45115. <https://doi.org/10.1371/journal.pone.0045115>.
- Liakhovitskaia, A., S. Rybtsov, T. Smith, A. Batsivari, N. Rybtsova, C. Rode, M. de Bruijn, et al. 2014. « Runx1 Is Required for Progression of CD41+ Embryonic Precursors into HSCs but Not Prior to This ». *Development* 141 (17): 3319-23. <https://doi.org/10.1242/dev.110841>.
- Ling, Kam-Wing, Katrin Ottersbach, Jan Piet van Hamburg, Aneta Oziemlak, Fong-Ying Tsai, Stuart H. Orkin, Rob Ploemacher, Rudi W. Hendriks, et Elaine Dzierzak. 2004. « GATA-2 Plays Two Functionally Distinct Roles during the Ontogeny of Hematopoietic Stem Cells ». *The Journal of Experimental Medicine* 200 (7): 871-82. <https://doi.org/10.1084/jem.20031556>.
- Ling-Hui, L, et J P Gergen. 1999. « Interactions between Brother, Runt and Lozenge ». *Development*, 10.
- Linzen, Ulrike, Richard Lilischkis, Ruwin Pandithage, Britta Schilling, Andrea Ullius, Juliane Lüscher-Firzlaff, Elisabeth Kremmer, Bernhard Lüscher, et Jörg Vervoorts. 2015. « ING5 Is Phosphorylated by CDK2 and Controls Cell Proliferation Independently of P53 ». Édité par Riccardo Alessandro. *PLoS ONE* 10 (4): e0123736. <https://doi.org/10.1371/journal.pone.0123736>.
- Liu, N., J. Wang, J. Wang, R. Wang, Z. Liu, Y. Yu, et H. Lu. 2013. « ING5 Is a Tip60 Cofactor That Acetylates P53 in Response to DNA Damage ». *Cancer Research* 73 (12): 3749-60. <https://doi.org/10.1158/0008-5472.CAN-12-3684>.
- Liu, Yizhou, Matthew D. Cheney, Justin J. Gaudet, Maksymilian Chruszcz, Stephen M. Lukasik, Daisuke Sugiyama, Jeff Lary, et al. 2006. « The Tetramer Structure of the Nervy Homology Two Domain, NHR2, Is Critical for AML1/ETO's Activity ». *Cancer Cell* 9 (4): 249-60. <https://doi.org/10.1016/j.ccr.2006.03.012>.

- Loke, Justin, Salam A. Assi, Maria Rosaria Imperato, Anetta Ptasinska, Pierre Cauchy, Yura Grabovska, Natalia Martinez Soria, et al. 2017. « RUNX1-ETO and RUNX1-EVI1 Differentially Reprogram the Chromatin Landscape in t(8;21) and t(3;21) AML ». *Cell Reports* 19 (8): 1654-68. <https://doi.org/10.1016/j.celrep.2017.05.005>.
- Lucitti, J. L., E. A. V. Jones, C. Huang, J. Chen, S. E. Fraser, et M. E. Dickinson. 2007. « Vascular Remodeling of the Mouse Yolk Sac Requires Hemodynamic Force ». *Development* 134 (18): 3317-26. <https://doi.org/10.1242/dev.02883>.
- Lv, Deguan, Feng Jia, Yanli Hou, Youzhou Sang, Angel A. Alvarez, Weiwei Zhang, Wei-Qiang Gao, et al. 2017. « Histone Acetyltransferase KAT6A Upregulates PI3K/AKT Signaling through TRIM24 Binding ». *Cancer Research* 77 (22): 6190-6201. <https://doi.org/10.1158/0008-5472.CAN-17-1388>.
- Makhijani, K., B. Alexander, T. Tanaka, E. Rulifson, et K. Bruckner. 2011. « The Peripheral Nervous System Supports Blood Cell Homing and Survival in the *Drosophila* Larva ». *Development* 138 (24): 5379-91. <https://doi.org/10.1242/dev.067322>.
- Makhijani, Kalpana, Brandy Alexander, Deepti Rao, Sophia Petraki, Leire Herboso, Katelyn Kukar, Itrat Batool, et al. 2017. « Regulation of *Drosophila* Hematopoietic Sites by Activin- β from Active Sensory Neurons ». *Nature Communications* 8 (1): 15990. <https://doi.org/10.1038/ncomms15990>.
- Mandal, Lolitika, Utpal Banerjee, et Volker Hartenstein. 2004. « Evidence for a Fruit Fly Hemangioblast and Similarities between Lymph-Gland Hematopoiesis in Fruit Fly and Mammal Aorta-Gonadal-Mesonephros Mesoderm ». *Nature Genetics* 36 (9): 1019-23. <https://doi.org/10.1038/ng1404>.
- Medvinsky, Alexander, et Elaine Dzierzak. 1996. « Definitive Hematopoiesis Is Autonomously Initiated by the AGM Region ». *Cell* 86 (6): 897-906. [https://doi.org/10.1016/S0092-8674\(00\)80165-8](https://doi.org/10.1016/S0092-8674(00)80165-8).
- Meng, Zhipeng, Toshiro Moroishi, et Kun-Liang Guan. 2016. « Mechanisms of Hippo Pathway Regulation ». *Genes & Development* 30 (1): 1-17. <https://doi.org/10.1101/gad.274027.115>.
- Mikkola, H. K. A. 2006. « The Journey of Developing Hematopoietic Stem Cells ». *Development* 133 (19): 3733-44. <https://doi.org/10.1242/dev.02568>.
- Miller, Marion, Aichun Chen, Vanessa Gobert, Benoit Augé, Mathilde Beau, Odile Burlet-Schiltz, Marc Haenlin, et Lucas Waltzer. 2017. « Control of RUNX-Induced Repression of Notch Signaling by MLF and Its Partner DnaJ-1 during *Drosophila* Hematopoiesis ». Édité par Claude Desplan. *PLOS Genetics* 13 (7): e1006932. <https://doi.org/10.1371/journal.pgen.1006932>.
- Milton, Claire C., Felix A. Grusche, Joffrey L. Degoutin, Eefang Yu, Qi Dai, Eric C. Lai, et Kieran F. Harvey. 2014. « The Hippo Pathway Regulates Hematopoiesis in *Drosophila* *Melanogaster* ». *Current Biology* 24 (22): 2673-80. <https://doi.org/10.1016/j.cub.2014.10.031>.
- Minakhina, Svetlana, William Tan, et Ruth Steward. 2011. « JAK/STAT and the GATA Factor Pannier Control Hemocyte Maturation and Differentiation in *Drosophila* ». *Developmental Biology* 352 (2): 308-16. <https://doi.org/10.1016/j.ydbio.2011.01.035>.

- Mitani, K., S. Ogawa, T. Tanaka, H. Miyoshi, M. Kurokawa, H. Mano, Y. Yazaki, M. Ohki, et H. Hirai. 1994. « Generation of the AML1-EVI-1 Fusion Gene in the t(3;21)(Q26;Q22) Causes Blastic Crisis in Chronic Myelocytic Leukemia. » *The EMBO Journal* 13 (3): 504-10. <https://doi.org/10.1002/j.1460-2075.1994.tb06288.x>.
- Miyoshi, Hiroyuki, et Misao Ohki. 1991. « T(8;21) Breakpoints on Chromosome 21 in Acute Myeloid Leukemia Are Clustered within a Limited Region of a Single Gene, AML1 », 4.
- Moeller, Morten E., Stanislav Nagy, Stephan U. Gerlach, Karen C. Soegaard, E. Thomas Danielsen, Michael J. Texada, et Kim F. Rewitz. 2017. « Warts Signaling Controls Organ and Body Growth through Regulation of Ecdysone ». *Current Biology* 27 (11): 1652-1659.e4. <https://doi.org/10.1016/j.cub.2017.04.048>.
- Mohammadi, Kiyannoush, Reza Safaralizadeh, Mohammadali Hosseinpour-Feizi, Narges Dastmalchi, et Yaghoub Moaddab. 2018. « Investigation of the Changes in the Expression Levels of MOZ Gene in Colorectal Cancer Tissues ». *Journal of Gastrointestinal Oncology* 10 (1): 68-73. <https://doi.org/10.21037/jgo.2018.09.12>.
- Morgan, T.H., C.B. Bridges, et A.H. Sturtevant. 1925. « The genetics of *Drosophila melanogaster* ». *Biblphia Genet.*
- Morrison, Sean J., et David T. Scadden. 2014. « The Bone Marrow Niche for Haematopoietic Stem Cells ». *Nature* 505 (7483): 327-34. <https://doi.org/10.1038/nature12984>.
- Mukherjee, Tina, William Sang Kim, Lolitika Mandal, et Utpal Banerjee. 2011. « Interaction Between Notch and Hif-a in Development and Survival of *Drosophila* Blood Cells » 332: 5.
- Muller, Paul Andrew, Balázs Koscsó, Gaurav Manohar Rajani, Korey Stevanovic, Marie-Luise Berres, Daigo Hashimoto, Arthur Mortha, et al. 2014. « Crosstalk between Muscularis Macrophages and Enteric Neurons Regulates Gastrointestinal Motility ». *Cell* 158 (2): 300-313. <https://doi.org/10.1016/j.cell.2014.04.050>.
- Nainu, Firzan, Yumiko Tanaka, Akiko Shiratsuchi, et Yoshinobu Nakanishi. 2015. « Protection of Insects against Viral Infection by Apoptosis-Dependent Phagocytosis ». *The Journal of Immunology* 195 (12): 5696-5706. <https://doi.org/10.4049/jimmunol.1500613>.
- Nakagawa, M., M. Ichikawa, K. Kumano, S. Goyama, M. Kawazu, T. Asai, S. Ogawa, M. Kurokawa, et S. Chiba. 2006. « AML1/Runx1 Rescues Notch1-Null Mutation-Induced Deficiency of Para-Aortic Splanchnopleural Hematopoiesis ». *Blood* 108 (10): 3329-34. <https://doi.org/10.1182/blood-2006-04-019570>.
- Nam, Hyuck-Jin, In-Hwan Jang, Hyejin You, Kyung-Ah Lee, et Won-Jae Lee. 2012. « Genetic Evidence of a Redox-Dependent Systemic Wound Response via Haya Protease-Phenoloxidase System in *Drosophila*: Redox-Modulated Systemic Wound Response ». *The EMBO Journal* 31 (5): 1253-65. <https://doi.org/10.1038/emboj.2011.476>.
- Nehme, Nadine T., Jessica Quintin, Ju Hyun Cho, Janice Lee, Marie-Céline Lafarge, Christine Kocks, et Dominique Ferrandon. 2011. « Relative Roles of the Cellular and Humoral Responses in the *Drosophila* Host Defense against Three Gram-Positive Bacterial Infections ». Édité par Nick Gay. *PLoS ONE* 6 (3): e14743. <https://doi.org/10.1371/journal.pone.0014743>.

- Ng, Cherry Ee Lin, Tomomasa Yokomizo, Namiko Yamashita, Branko Cirovic, Hao Jin, Zilong Wen, Yoshiaki Ito, et Motomi Osato. 2010. « A Runx1 Intronic Enhancer Marks Hemogenic Endothelial Cells and Hematopoietic Stem Cells ». *STEM CELLS* 28 (10): 1869-81. <https://doi.org/10.1002/stem.507>.
- Niki, M., H. Okada, H. Takano, J. Kuno, K. Tani, H. Hibino, S. Asano, Y. Ito, M. Satake, et T. Noda. 1997. « Hematopoiesis in the Fetal Liver Is Impaired by Targeted Mutagenesis of a Gene Encoding a Non-DNA Binding Subunit of the Transcription Factor, Polyomavirus Enhancer Binding Protein 2/Core Binding Factor ». *Proceedings of the National Academy of Sciences* 94 (11): 5697-5702. <https://doi.org/10.1073/pnas.94.11.5697>.
- North, Trista E, Marella F.T.R de Bruijn, Terryl Stacy, Laleh Talebian, Evan Lind, Catherine Robin, Michael Binder, Elaine Dzierzak, et Nancy A Speck. 2002. « Runx1 Expression Marks Long-Term Repopulating Hematopoietic Stem Cells in the Midgestation Mouse Embryo ». *Immunity* 16 (5): 661-72. [https://doi.org/10.1016/S1074-7613\(02\)00296-0](https://doi.org/10.1016/S1074-7613(02)00296-0).
- North, Trista, Ting-Lei Gu, Terryl Stacy, Qing Wang, Louisa Howard, Michael Binder, Miguel Marín-Padilla, et Nancy A Speck. 1999. « Cbfa2 Is Required for the Formation of Intra-Aortic Hematopoietic Clusters », 13.
- Nottingham, W. T., A. Jarratt, M. Burgess, C. L. Speck, J.-F. Cheng, S. Prabhakar, E. M. Rubin, et al. 2007. « Runx1-Mediated Hematopoietic Stem-Cell Emergence Is Controlled by a Gata/Ets/SCL-Regulated Enhancer ». *Blood* 110 (13): 4188-97. <https://doi.org/10.1182/blood-2007-07-100883>.
- Nucifora, Giuseppina, Catherine R Begy, Hirofumi Kobayashi, Diane Roulston, DAVID CLAXTONt, JENS PEDERSEN-BJERGAARDt, Evan Parganas, James N Ihle, et Janet D Rowley. 1994. « Consistent Intergenic Splicing and Production of Multiple Transcripts between AML1 at 21q22 and Unrelated Genes at 3q26 in (3;21)(Q26;Q22) Translocations », 5.
- Nukina, Arika, Yuki Kagoya, Naoko Watanabe-Okochi, Shunya Arai, Koki Ueda, Akihide Yoshimi, Yasuhito Nannya, et Mineo Kurokawa. 2014. « Single-Cell Gene Expression Analysis Reveals Clonal Architecture of Blast-Phase Chronic Myeloid Leukaemia ». *British Journal of Haematology* 165 (3): 414-16. <https://doi.org/10.1111/bjh.12726>.
- Okuda, Tsukasa, Zhongling Cai, Shouli Yang, Noel Lenny, Chuhi-joo Lyu, Hironori Harada, et James R Downing. 1998. « Expression of a Knocked-In AML1-ETO Leukemia Gene Inhibits the Establishment of Normal Definitive Hematopoiesis and Directly Generates Dysplastic Hematopoietic Progenitors », 11.
- Okuda, Tsukasa, Jan van Deursen, Scott W Hiebert, Gerard Grosveld, et James R Downing. 1996. « AML1, the Target of Multiple Chromosomal Translocations in Human Leukemia, Is Essential for Normal Fetal Liver Hematopoiesis ». *Cell* 84 (2): 321-30. [https://doi.org/10.1016/S0092-8674\(00\)80986-1](https://doi.org/10.1016/S0092-8674(00)80986-1).
- Olofsson, Birgitta, et Damon T. Page. 2005. « Condensation of the Central Nervous System in Embryonic *Drosophila* Is Inhibited by Blocking Hemocyte Migration or Neural Activity ». *Developmental Biology* 279 (1): 233-43. <https://doi.org/10.1016/j.ydbio.2004.12.020>.
- Orkin, Stuart H., et Leonard I. Zon. 2008. « Hematopoiesis: An Evolving Paradigm for Stem Cell Biology ». *Cell* 132 (4): 631-44. <https://doi.org/10.1016/j.cell.2008.01.025>.

- Ormaza, Georgina, Jhon A. Rodríguez, Alain Ibáñez de Opakua, Nekane Merino, Maider Villate, Irantzu Gorroño, Miriam Rábano, et al. 2019. « The Tumor Suppressor ING5 Is a Dimeric, Bivalent Recognition Molecule of the Histone H3K4me3 Mark ». *Journal of Molecular Biology*, avril, S0022283619302141. <https://doi.org/10.1016/j.jmb.2019.04.018>.
- Osman, D., V. Gobert, F. Ponthan, O. Heidenreich, M. Haenlin, et L. Waltzer. 2009. « A *Drosophila* Model Identifies Calpains as Modulators of the Human Leukemogenic Fusion Protein AML1-ETO ». *Proceedings of the National Academy of Sciences* 106 (29): 12043-48. <https://doi.org/10.1073/pnas.0902449106>.
- Ota, M., et H. Sasaki. 2008. « Mammalian Tead Proteins Regulate Cell Proliferation and Contact Inhibition as Transcriptional Mediators of Hippo Signaling ». *Development* 135 (24): 4059-69. <https://doi.org/10.1242/dev.027151>.
- Oyallon, Justine, Nathalie Vanzo, Joanna Krzemień, Ismaël Morin-Poulard, Alain Vincent, et Michèle Crozatier. 2016. « Two Independent Functions of Collier/Early B Cell Factor in the Control of *Drosophila* Blood Cell Homeostasis ». Édité par Kenneth Söderhäll. *PLOS ONE* 11 (2): e0148978. <https://doi.org/10.1371/journal.pone.0148978>.
- Palis, J. 1999. « Development of Hematopoietic Progenitors », 12.
- Panagopoulos, I. 2001. « Fusion of the MORF and CBP Genes in Acute Myeloid Leukemia with the t(10;16)(Q22;P13) ». *Human Molecular Genetics* 10 (4): 395-404. <https://doi.org/10.1093/hmg/10.4.395>.
- Papp, B. 2006. « Histone Trimethylation and the Maintenance of Transcriptional ON and OFF States by TrxG and PcG Proteins ». *Genes & Development* 20 (15): 2041-54. <https://doi.org/10.1101/gad.388706>.
- Patel, Jay P., Mithat Gönen, Maria E. Figueroa, Hugo Fernandez, Zhuoxin Sun, Janis Racevskis, Pieter Van Vlierberghe, et al. 2012. « Prognostic Relevance of Integrated Genetic Profiling in Acute Myeloid Leukemia ». *New England Journal of Medicine* 366 (12): 1079-89. <https://doi.org/10.1056/NEJMoa1112304>.
- Pater, Emma de, Polynikis Kaimakis, Chris S. Vink, Tomomasa Yokomizo, Tomoko Yamada-Inagawa, Reinier van der Linden, Parham Solaimani Kartalaei, Sally A. Camper, Nancy Speck, et Elaine Dzierzak. 2013. « *Gata2* Is Required for HSC Generation and Survival ». *The Journal of Experimental Medicine* 210 (13): 2843-50. <https://doi.org/10.1084/jem.20130751>.
- Peebles, EE, A Geisler, CJ Whitcraft, et CP Oliver. 1969. « Activity of phenol oxidases at the puparium formation stage in development of nineteen lozenge mutants of *Drosophila melanogaster* ». *Biochem Genet*.
- Pencovich, N., R. Jaschek, A. Tanay, et Y. Groner. 2011. « Dynamic Combinatorial Interactions of RUNX1 and Cooperating Partners Regulates Megakaryocytic Differentiation in Cell Line Models ». *Blood* 117 (1): e1-14. <https://doi.org/10.1182/blood-2010-07-295113>.
- Perez-Campo, F. M., J. Borrow, V. Kouskoff, et G. Lacaud. 2009. « The Histone Acetyl Transferase Activity of Monocytic Leukemia Zinc Finger Is Critical for the Proliferation of Hematopoietic Precursors ». *Blood* 113 (20): 4866-74. <https://doi.org/10.1182/blood-2008-04-152017>.

- Perez-Campo, Flor M., Guilherme Costa, Michael Lie-a-Ling, Stefano Stifani, Valerie Kouskoff, et Georges Lacaud. 2014. « MOZ-Mediated Repression of *P16^{INK4a}* Is Critical for the Self-Renewal of Neural and Hematopoietic Stem Cells: MOZ: Key Regulator of Proliferation versus Senescence ». *STEM CELLS* 32 (6): 1591-1601. <https://doi.org/10.1002/stem.1606>.
- Pippa, Raffaella, Ana Dominguez, Raquel Malumbres, Akinori Endo, Elena Arriazu, Nerea Marcotegui, Elizabeth Guruceaga, et María D. Odero. 2017. « MYC-Dependent Recruitment of RUNX1 and GATA2 on the SET Oncogene Promoter Enhances PP2A Inactivation in Acute Myeloid Leukemia ». *Oncotarget* 8 (33). <https://doi.org/10.18632/oncotarget.9840>.
- Poplawski, Amanda, Kaifeng Hu, Woonghee Lee, Senthil Natesan, Danni Peng, Samuel Carlson, Xiaobing Shi, Stefan Balaz, John L. Markley, et Karen C. Glass. 2014. « Molecular Insights into the Recognition of N-Terminal Histone Modifications by the BRPF1 Bromodomain ». *Journal of Molecular Biology* 426 (8): 1661-76. <https://doi.org/10.1016/j.jmb.2013.12.007>.
- Ptasinska, Anetta, Salam A. Assi, Natalia Martinez-Soria, Maria Rosaria Imperato, Jason Piper, Pierre Cauchy, Anna Pickin, et al. 2014. « Identification of a Dynamic Core Transcriptional Network in t(8;21) AML That Regulates Differentiation Block and Self-Renewal ». *Cell Reports* 8 (6): 1974-88. <https://doi.org/10.1016/j.celrep.2014.08.024>.
- Qiu, C., E. N. Olivier, M. Velho, et E. E. Bouhassira. 2008. « Globin Switches in Yolk Sac-like Primitive and Fetal-like Definitive Red Blood Cells Produced from Human Embryonic Stem Cells ». *Blood* 111 (4): 2400-2408. <https://doi.org/10.1182/blood-2007-07-102087>.
- Rebel, V. I., A. L. Kung, E. A. Tanner, H. Yang, R. T. Bronson, et D. M. Livingston. 2002. « Distinct Roles for CREB-Binding Protein and P300 in Hematopoietic Stem Cell Self-Renewal ». *Proceedings of the National Academy of Sciences* 99 (23): 14789-94. <https://doi.org/10.1073/pnas.232568499>.
- Regan, Jennifer C., Ana S. Brandão, Alexandre B. Leitão, Ângela Raquel Mantas Dias, Élio Sucena, António Jacinto, et Anna Zaidman-Rémy. 2013. « Steroid Hormone Signaling Is Essential to Regulate Innate Immune Cells and Fight Bacterial Infection in *Drosophila* ». Édité par David S. Schneider. *PLoS Pathogens* 9 (10): e1003720. <https://doi.org/10.1371/journal.ppat.1003720>.
- Rehorn, Klaus-Peter, Heidi Thelen, Alan M Michelson, et Rolf Reuter. 1996. « A Molecular Aspect of Hematopoiesis and Endoderm Development Common to Vertebrates and *Drosophila* », 9.
- Reiter, L. T. 2001. « A Systematic Analysis of Human Disease-Associated Gene Sequences In *Drosophila Melanogaster* ». *Genome Research* 11 (6): 1114-25. <https://doi.org/10.1101/gr.169101>.
- Rennert, Jessica, James A Coffman, Arcady R Mushegian, et Anthony J Robertson. 2003. « The Evolution of Runx Genes I. A Comparative Study of Sequences from Phylogenetically Diverse Model Organisms ». *BMC Evolutionary Biology*, 11.
- Richard, Charlotte, Cécile Drevon, Pierre-Yves Canto, Gaelle Villain, Karine Bollérot, Aveline Lempereur, Marie-Aimée Teillet, et al. 2013. « Endothelio-Mesenchymal Interaction

- Controls Runx1 Expression and Modulates the Notch Pathway to Initiate Aortic Hematopoiesis ». *Developmental Cell* 24 (6): 600-611. <https://doi.org/10.1016/j.devcel.2013.02.011>.
- Rizki, M. T. M. 1957. « Alterations in the Haemocyte Population of *Drosophila Melanogaster* ». *Journal of Morphology* 100 (3): 437-58. <https://doi.org/10.1002/jmor.1051000303>.
- Rizki, T.M., et R.M. Rizki. 1981. « Alleles of lz as suppressors of the Bc-phenotype in *Drosophila melanogaster* ». *Genetics*.
- . 1992. « Lamellocyte Differentiation in *Drosophila* Larvae Parasitized by Leptopilina ». *Developmental & Comparative Immunology* 16 (2-3): 103-10. [https://doi.org/10.1016/0145-305X\(92\)90011-Z](https://doi.org/10.1016/0145-305X(92)90011-Z).
- Robert-Moreno, A. 2005. « RBPj -Dependent Notch Function Regulates Gata2 and Is Essential for the Formation of Intra-Embryonic Hematopoietic Cells ». *Development* 132 (5): 1117-26. <https://doi.org/10.1242/dev.01660>.
- Robert-Moreno, Àlex, Jordi Guiu, Cristina Ruiz-Herguido, M Eugenia López, Julia Inglés-Esteve, Lluís Riera, Alex Tipping, et al. 2008. « Impaired Embryonic Haematopoiesis yet Normal Arterial Development in the Absence of the Notch Ligand Jagged1 ». *The EMBO Journal* 27 (13): 1886-95. <https://doi.org/10.1038/emboj.2008.113>.
- Rokudai, S., O. Laptchenko, S. M. Arnal, Y. Taya, I. Kitabayashi, et C. Prives. 2013. « MOZ Increases P53 Acetylation and Premature Senescence through Its Complex Formation with PML ». *Proceedings of the National Academy of Sciences* 110 (10): 3895-3900. <https://doi.org/10.1073/pnas.1300490110>.
- Rokudai, Susumu, Yukiko Aikawa, Yusuke Tagata, Nobuo Tsuchida, Yoichi Taya, et Issay Kitabayashi. 2009. « Monocytic Leukemia Zinc Finger (MOZ) Interacts with P53 to Induce P21 Expression and Cell-Cycle Arrest ». *Journal of Biological Chemistry* 284 (1): 237-44. <https://doi.org/10.1074/jbc.M805101200>.
- Rothenberg, E. V. 2013. « GATA-3 Locks the Door to the B-Cell Option ». *Blood* 121 (10): 1673-74. <https://doi.org/10.1182/blood-2013-01-477737>.
- Roudaia, L., M. D. Cheney, E. Manuylova, W. Chen, M. Morrow, S. Park, C.-T. Lee, et al. 2009. « CBF Is Critical for AML1-ETO and TEL-AML1 Activity ». *Blood* 113 (13): 3070-79. <https://doi.org/10.1182/blood-2008-03-147207>.
- Sabapathy, Kanaga, et David P Lane. 2019. « Understanding P53 Functions through P53 Antibodies ». Édité par Chandra S Verma. *Journal of Molecular Cell Biology* 11 (4): 317-29. <https://doi.org/10.1093/jmcb/mjz010>.
- Salat, D., R. Liefke, J. Wiedenmann, T. Borggrefe, et F. Oswald. 2008. « ETO, but Not Leukemogenic Fusion Protein AML1/ETO, Augments RBP-J /SHARP-Mediated Repression of Notch Target Genes ». *Molecular and Cellular Biology* 28 (10): 3502-12. <https://doi.org/10.1128/MCB.01966-07>.
- Sapountzi, Vasileia, et Jacques Côté. 2011. « MYST-Family Histone Acetyltransferases: Beyond Chromatin ». *Cellular and Molecular Life Sciences* 68 (7): 1147-56. <https://doi.org/10.1007/s00018-010-0599-9>.

- Schulz, Christian, Elisa Gomez Perdiguero, Laurent Chorro, Heather Szabo-Rogers, Nicolas Cagnard, Katrin Kierdorf, Marco Prinz, et al. 2012. « A Lineage of Myeloid Cells Independent of Myb and Hematopoietic Stem Cells ». *Science* 336 (6077): 86-90. <https://doi.org/10.1126/science.1219179>.
- Scott, Ethan K., Tzumin Lee, et Liqun Luo. 2001. « Enok Encodes a *Drosophila* Putative Histone Acetyltransferase Required for Mushroom Body Neuroblast Proliferation ». *Current Biology* 11 (2): 99-104. [https://doi.org/10.1016/S0960-9822\(01\)00020-3](https://doi.org/10.1016/S0960-9822(01)00020-3).
- Sheikh, B. N., Y. Yang, J. Schreuder, S. K. Nilsson, R. Bilardi, S. Carotta, H. M. McRae, D. Metcalf, A. K. Voss, et T. Thomas. 2016. « MOZ (KAT6A) Is Essential for the Maintenance of Classically Defined Adult Hematopoietic Stem Cells ». *Blood* 128 (19): 2307-18. <https://doi.org/10.1182/blood-2015-10-676072>.
- Sheikh, Bilal N., Natalie L. Downer, Belinda Phipson, Hannah K. Vanyai, Andrew J. Kueh, Davis J. McCarthy, Gordon K. Smyth, Tim Thomas, et Anne K. Voss. 2015. « MOZ and BMI1 Play Opposing Roles during *Hox* Gene Activation in ES Cells and in Body Segment Identity Specification in Vivo ». *Proceedings of the National Academy of Sciences* 112 (17): 5437-42. <https://doi.org/10.1073/pnas.1422872112>.
- Sheikh, Bilal N., Donald Metcalf, Anne K. Voss, et Tim Thomas. 2017. « MOZ and BMI1 Act Synergistically to Maintain Hematopoietic Stem Cells ». *Experimental Hematology* 47 (mars): 83-97.e8. <https://doi.org/10.1016/j.exphem.2016.10.006>.
- Sinenko, S. A., T. Hung, T. Moroz, Q.-M. Tran, S. Sidhu, M. D. Cheney, N. A. Speck, et U. Banerjee. 2010. « Genetic Manipulation of AML1-ETO-Induced Expansion of Hematopoietic Precursors in a *Drosophila* Model ». *Blood* 116 (22): 4612-20. <https://doi.org/10.1182/blood-2010-03-276998>.
- Skalska, L., R. Stojnic, J. Li, B. Fischer, G. Cerda-Moya, H. Sakai, S. Tajbakhsh, S. Russell, B. Adryan, et S. J. Bray. 2015. « Chromatin Signatures at Notch-Regulated Enhancers Reveal Large-Scale Changes in H3K56ac upon Activation ». *The EMBO Journal* 34 (14): 1889-1904. <https://doi.org/10.15252/embj.201489923>.
- Spahn, Philipp, Sven Huelsmann, Klaus-Peter Rehorn, Stefanie Mischke, Melanie Mayer, Andreu Casali, et Rolf Reuter. 2014. « Multiple Regulatory Safeguards Confine the Expression of the GATA Factor Serpent to the Hemocyte Primordium within the *Drosophila* Mesoderm ». *Developmental Biology* 386 (1): 272-79. <https://doi.org/10.1016/j.ydbio.2013.12.012>.
- Spangrude, Gerald J, Shelly Heimfeld, et Irving L Weissman. 1988. « Purification and Characterization of Mouse Hematopoietic Stem Cells », 6.
- Stofanko, Martin, So Yeon Kwon, et Paul Badenhorst. 2010. « Lineage Tracing of Lamellocytes Demonstrates *Drosophila* Macrophage Plasticity ». Édité par Francois Schweisguth. *PLoS ONE* 5 (11): e14051. <https://doi.org/10.1371/journal.pone.0014051>.
- Sun, Cheng-Cao, Shu-Jun Li, Zhen-Long Chen, Guang Li, Qian Zhang, et De-Jia Li. 2019. « Expression and Prognosis Analyses of Runt-Related Transcription Factor Family in Human Leukemia ». *Molecular Therapy - Oncolytics* 12 (mars): 103-11. <https://doi.org/10.1016/j.omto.2018.12.008>.

- Sun, Xiao-Jian, Na Man, Yurong Tan, Stephen D. Nimer, et Lan Wang. 2015. « The Role of Histone Acetyltransferases in Normal and Malignant Hematopoiesis ». *Frontiers in Oncology* 5 (mai). <https://doi.org/10.3389/fonc.2015.00108>.
- Sun, Xiao-Jian, Zhanxin Wang, Lan Wang, Yanwen Jiang, Nils Kost, T. David Soong, Wei-Yi Chen, et al. 2013. « A Stable Transcription Factor Complex Nucleated by Oligomeric AML1-ETO Controls Leukaemogenesis ». *Nature* 500 (7460): 93-97. <https://doi.org/10.1038/nature12287>.
- Takarada, Takeshi, Ryota Nakazato, Azusa Tsuchikane, Koichi Fujikawa, Takashi Iezaki, Yukio Yoneda, et Eiichi Hinoi. 2016. « Genetic Analysis of Runx2 Function during Intramembranous Ossification ». *Development* 143 (2): 211-18. <https://doi.org/10.1242/dev.128793>.
- Tavian, Manuela, et Bruno Peault. 2005. « Embryonic Development of the Human Hematopoietic System ». *The International Journal of Developmental Biology* 49 (2-3): 243-50. <https://doi.org/10.1387/ijdb.041957mt>.
- Tepass, Ulrich, Liselotte I Fessler, Amina Aziz, et Volker Hartenstein. 1994. « Embryonic Origin of Hemocytes and Their Relationship to Cell Death in *Drosophila* », 9.
- Terriente-Felix, A., J. Li, S. Collins, A. Mulligan, I. Reekie, F. Bernard, A. Krejci, et S. Bray. 2013. « Notch Cooperates with Lozenge/Runx to Lock Haemocytes into a Differentiation Programme ». *Development* 140 (4): 926-37. <https://doi.org/10.1242/dev.086785>.
- Tham, Emma, Anna Lindstrand, Avni Santani, Helena Malmgren, Addie Nesbitt, Holly A. Dubbs, Elaine H. Zackai, et al. 2015. « Dominant Mutations in KAT6A Cause Intellectual Disability with Recognizable Syndromic Features ». *The American Journal of Human Genetics* 96 (3): 507-13. <https://doi.org/10.1016/j.ajhg.2015.01.016>.
- The Cancer Genome Atlas Research Network. 2013. « Genomic and Epigenomic Landscapes of Adult De Novo Acute Myeloid Leukemia ». *New England Journal of Medicine* 368 (22): 2059-74. <https://doi.org/10.1056/NEJMoa1301689>.
- Thomas, T. 2006. « Monocytic Leukemia Zinc Finger Protein Is Essential for the Development of Long-Term Reconstituting Hematopoietic Stem Cells ». *Genes & Development* 20 (9): 1175-86. <https://doi.org/10.1101/gad.1382606>.
- Tijssen, Marloes R., Ana Cvejic, Anagha Joshi, Rebecca L. Hannah, Rita Ferreira, Ariel Forrai, Dana C. Bellissimo, et al. 2011. « Genome-Wide Analysis of Simultaneous GATA1/2, RUNX1, FLI1, and SCL Binding in Megakaryocytes Identifies Hematopoietic Regulators ». *Developmental Cell* 20 (5): 597-609. <https://doi.org/10.1016/j.devcel.2011.04.008>.
- Tober, J., A. Koniski, K. E. McGrath, R. Vemishetti, R. Emerson, K. K. L. de Mesy-Bentley, R. Waugh, et J. Palis. 2007. « The Megakaryocyte Lineage Originates from Hemangioblast Precursors and Is an Integral Component Both of Primitive and of Definitive Hematopoiesis ». *Blood* 109 (4): 1433-41. <https://doi.org/10.1182/blood-2006-06-031898>.
- Travnickova, Jana, Vanessa Tran Chau, Emmanuelle Julien, Julio Mateos-Langerak, Catherine Gonzalez, Etienne Lelièvre, Georges Lutfalla, Manuela Tavian, et Karima Kissa. 2015.

- « Primitive Macrophages Control HSPC Mobilization and Definitive Haematopoiesis ». *Nature Communications* 6 (1): 6227. <https://doi.org/10.1038/ncomms7227>.
- Tsai, Fong-Ying, Gordon Keller, Frank C. Kuo, Mitchell Weiss, Jianzhou Chen, Margery Rosenblatt, Frederick W. Alt, et Stuart H. Orkin. 1994. « An Early Haematopoietic Defect in Mice Lacking the Transcription Factor GATA-2 ». *Nature* 371 (6494): 221-26. <https://doi.org/10.1038/371221a0>.
- Uderhardt, Stefan, Andrew J. Martins, John S. Tsang, Tim Lämmermann, et Ronald N. Germain. 2019. « Resident Macrophages Cloak Tissue Microlesions to Prevent Neutrophil-Driven Inflammatory Damage ». *Cell* 177 (3): 541-555.e17. <https://doi.org/10.1016/j.cell.2019.02.028>.
- Ullah, M., N. Pelletier, L. Xiao, S. P. Zhao, K. Wang, C. Degerny, S. Tahmasebi, et al. 2008. « Molecular Architecture of Quartet MOZ/MORF Histone Acetyltransferase Complexes ». *Molecular and Cellular Biology* 28 (22): 6828-43. <https://doi.org/10.1128/MCB.01297-08>.
- Vogeli, Kevin M., Suk-Won Jin, Gail R. Martin, et Didier Y. R. Stainier. 2006. « A common progenitor for haematopoietic and endothelial lineages in the zebrafish gastrula ». *Nature* 443 (7109): 337-39. <https://doi.org/10.1038/nature05045>.
- Voss, Anne K., Caitlin Collin, Mathew P. Dixon, et Tim Thomas. 2009. « Moz and Retinoic Acid Coordinately Regulate H3K9 Acetylation, Hox Gene Expression, and Segment Identity ». *Developmental Cell* 17 (5): 674-86. <https://doi.org/10.1016/j.devcel.2009.10.006>.
- Waltzer, L. 2002. « Two Isoforms of Serpent Containing Either One or Two GATA Zinc Fingers Have Different Roles in *Drosophila* Haematopoiesis ». *The EMBO Journal* 21 (20): 5477-86. <https://doi.org/10.1093/emboj/cdf545>.
- . 2003a. « Cooperation between the GATA and RUNX Factors Serpent and Lozenge during *Drosophila* Hematopoiesis ». *The EMBO Journal* 22 (24): 6516-25. <https://doi.org/10.1093/emboj/cdg622>.
- . 2003b. « Cooperation between the GATA and RUNX Factors Serpent and Lozenge during *Drosophila* Hematopoiesis ». *The EMBO Journal* 22 (24): 6516-25. <https://doi.org/10.1093/emboj/cdg622>.
- Waltzer, Lucas, Vanessa Gobert, Dani Osman, et Marc Haenlin. 2010. « Transcription Factor Interplay during *Drosophila* Haematopoiesis ». *The International Journal of Developmental Biology* 54 (6-7): 1107-15. <https://doi.org/10.1387/ijdb.093054lw>.
- Wang, Dapeng, Huitian Diao, Adam J. Getzler, Walter Rogal, Megan A. Frederick, Justin Milner, Bingfei Yu, Shane Crotty, Ananda W. Goldrath, et Matthew E. Pipkin. 2018. « The Transcription Factor Runx3 Establishes Chromatin Accessibility of Cis-Regulatory Landscapes That Drive Memory Cytotoxic T Lymphocyte Formation ». *Immunity* 48 (4): 659-674.e6. <https://doi.org/10.1016/j.immuni.2018.03.028>.
- Wang, Yue-Ying, Guang-Biao Zhou, Tong Yin, Bing Chen, Jing-Yi Shi, Wen-Xue Liang, Xiao-Long Jin, et al. 2005. « AML1-ETO and C-KIT Mutation overexpression in t(8;21) Leukemia: Implication in Stepwise Leukemogenesis and Response to Gleevec », 6.
- Warner, CK, EH Grell, et KB Jacobson. 1974. « Phenol oxidase activity and the lozenge locus of *Drosophila melanogaster* ». *Biochem Genet.*

- Weiskopf, Kipp. 2016. « Myeloid Cell Origins, Differentiation, and Clinical Implications ». *Microbiology Spectrum* 4 (5). <https://doi.org/10.1128/microbiolspec.MCHD-0031-2016>.
- Wilson, Nicola K., Samuel D. Foster, Xiaonan Wang, Kathy Knezevic, Judith Schütte, Polynikis Kaimakis, Paulina M. Chilarska, et al. 2010. « Combinatorial Transcriptional Control In Blood Stem/Progenitor Cells: Genome-Wide Analysis of Ten Major Transcriptional Regulators ». *Cell Stem Cell* 7 (4): 532-44. <https://doi.org/10.1016/j.stem.2010.07.016>.
- Wood, Will, Celia Faria, et Antonio Jacinto. 2006. « Distinct Mechanisms Regulate Hemocyte Chemotaxis during Development and Wound Healing in *Drosophila Melanogaster* ». *The Journal of Cell Biology* 173 (3): 405-16. <https://doi.org/10.1083/jcb.200508161>.
- Wood, Will, et Paul Martin. 2017. « Macrophage Functions in Tissue Patterning and Disease: New Insights from the Fly ». *Developmental Cell* 40 (3): 221-33. <https://doi.org/10.1016/j.devcel.2017.01.001>.
- Woolf, E., C. Xiao, O. Fainaru, J. Lotem, D. Rosen, V. Negreanu, Y. Bernstein, et al. 2003. « Runx3 and Runx1 Are Required for CD8 T Cell Development during Thymopoiesis ». *Proceedings of the National Academy of Sciences* 100 (13): 7731-36. <https://doi.org/10.1073/pnas.1232420100>.
- Wu, Xiaochong, Paul A. Northcott, Adrian Dubuc, Adam J. Dupuy, David J. H. Shih, Hendrik Witt, Sidney Croul, et al. 2012. « Clonal Selection Drives Genetic Divergence of Metastatic Medulloblastoma ». *Nature* 482 (7386): 529-33. <https://doi.org/10.1038/nature10825>.
- Xie, Huafeng, Min Ye, Ru Feng, et Thomas Graf. 2004. « Stepwise Reprogramming of B Cells into Macrophages », 14.
- Xie, Wei, Sa A. Wang, C. Cameron Yin, Jie Xu, Shaoying Li, Carlos E. Bueso-Ramos, L. Jeffrey Medeiros, et Guilin Tang. 2019. « Acute Myeloid Leukemia with t(8;21)(Q22;Q22.1)/RUNX1-RUNX1T1 and KIT Exon 8 Mutation Is Associated with Characteristic Mastocytosis and Dismal Outcomes ». *Experimental and Molecular Pathology* 108 (juin): 131-36. <https://doi.org/10.1016/j.yexmp.2019.04.009>.
- Xin, Tianchi, Tao Xuan, Jieqiong Tan, Mengjie Li, Gengchun Zhao, et Mingfa Li. 2013. « The *Drosophila* Putative Histone Acetyltransferase Enok Maintains Female Germline Stem Cells through Regulating Bruno and the Niche ». *Developmental Biology* 384 (1): 1-12. <https://doi.org/10.1016/j.ydbio.2013.10.001>.
- Xiong, Xiaozhe, Tatyana Panchenko, Shuang Yang, Shuai Zhao, Peiqiang Yan, Wenhao Zhang, Wei Xie, et al. 2016. « Selective Recognition of Histone Crotonylation by Double PHD Fingers of MOZ and DPF2 ». *Nature Chemical Biology* 12 (12): 1111-18. <https://doi.org/10.1038/nchembio.2218>.
- Yamada, Osamu, et Kiyotaka Kawauchi. 2013. « The Role of the JAK-STAT Pathway and Related Signal Cascades in Telomerase Activation during the Development of Hematologic Malignancies ». *JAK-STAT* 2 (4): e25256. <https://doi.org/10.4161/jkst.25256>.
- Yang, X.-J. 2004. « The Diverse Superfamily of Lysine Acetyltransferases and Their Roles in Leukemia and Other Diseases ». *Nucleic Acids Research* 32 (3): 959-76. <https://doi.org/10.1093/nar/gkh252>.

- Yoshida, Hitoshi, et Issay Kitabayashi. 2008. « Chromatin Regulation by AML1 Complex ». *International Journal of Hematology* 87 (1): 19-24. <https://doi.org/10.1007/s12185-007-0004-0>.
- Yoshimoto, M., E. Montecino-Rodriguez, M. J. Ferkowicz, P. Porayette, W. C. Shelley, S. J. Conway, K. Dorshkind, et M. C. Yoder. 2011. « Embryonic Day 9 Yolk Sac and Intra-Embryonic Hemogenic Endothelium Independently Generate a B-1 and Marginal Zone Progenitor Lacking B-2 Potential ». *Proceedings of the National Academy of Sciences* 108 (4): 1468-73. <https://doi.org/10.1073/pnas.1015841108>.
- Yoshimoto, M., P. Porayette, N. L. Glosson, S. J. Conway, N. Carlesso, A. A. Cardoso, M. H. Kaplan, et M. C. Yoder. 2012. « Autonomous Murine T-Cell Progenitor Production in the Extra-Embryonic Yolk Sac before HSC Emergence ». *Blood* 119 (24): 5706-14. <https://doi.org/10.1182/blood-2011-12-397489>.
- You, Linya, Lin Li, Jinfeng Zou, Kezhi Yan, Jad Belle, Anastasia Nijnik, Edwin Wang, et Xiang-Jiao Yang. 2016. « BRPF1 Is Essential for Development of Fetal Hematopoietic Stem Cells ». *Journal of Clinical Investigation* 126 (9): 3247-62. <https://doi.org/10.1172/JCI80711>.
- Yu, Junyan, Xiangyang Tian, Jianlan Chang, Ping Liu, et Rong Zhang. 2017. « RUNX3 Inhibits the Proliferation and Metastasis of Gastric Cancer through Regulating MiR-182/HOXA9 ». *Biomedicine & Pharmacotherapy* 96 (décembre): 782-91. <https://doi.org/10.1016/j.biopha.2017.08.144>.
- Yzaguirre, Amanda D., Elizabeth D. Howell, Yan Li, Zijing Liu, et Nancy A. Speck. 2018. « Runx1 Is Sufficient for Blood Cell Formation from Non-Hemogenic Endothelial Cells *in Vivo* Only during Early Embryogenesis ». *Development* 145 (2): dev158162. <https://doi.org/10.1242/dev.158162>.
- Zaidan, Nada, et Katrin Ottersbach. 2018. « The Multi-Faceted Role of Gata3 in Developmental Haematopoiesis ». *Open Biology* 8 (11): 180152. <https://doi.org/10.1098/rsob.180152>.

L'hématopoïèse est un processus très finement régulé qui mène à la formation de chaque cellule sanguine d'un organisme. Chez les mammifères il existe un nombre important de types cellulaires qui participent à l'établissement des mécanismes de défense du corps. Toutes ces cellules proviennent de la différenciation terminale d'une cellule unique appelée Cellule Souche Hématopoïétique (CSH) qui, par le biais de différenciations successives donnant naissance à des progéniteurs de plus en plus spécifiés, va permettre l'établissement normal de tous les types de cellules sanguines. Les CSH sont parmi les cellules les plus finement contrôlées de l'organisme, car en effet une dérégulation de leur fonctionnement normal (prolifération excessive, différenciation prématurée...) peut entraîner de graves conséquences, à savoir des maladies du sang appelées leucémies.

De nombreux facteurs moléculaires sont impliqués dans la régulation des CSH, et certains d'entre eux sont les cibles de mutations ou réarrangements chromosomiques à l'origine de leucémies, tels que le facteur de transcription RUNX1 et la Lysine Acétyl-Transférase (KAT) Monocytic Leukemia Zinc-Finger (MOZ).

Chez la Drosophile, les cellules sanguines sont apparentées au lignage myéloïde des mammifères, et les acteurs moléculaires contrôlant leur formation sont très conservés. Ainsi, les cellules à cristaux (CC), qui sont les homologues fonctionnels des mégakaryocytes, sont formées suite à l'action conjointe de l'homologue de RUNX1, Lozenge (Lz) et de GATA1, Serpent (Srp). Un crible pan génomique mené par mon équipe d'accueil visant à trouver des modulateurs de l'activité transcriptionnelle de Lz et Srp, a permis de d'identifier le gène *enoki mushroom* (*enok*) comme étant un régulateur négatif de cette activité. Enok est l'homologue chez la Drosophile de MOZ, et une étude préliminaire de son rôle *in vivo* a pu mettre en évidence une fonction essentielle dans le développement des CC, qui disparaissent quasiment en totalité dans un contexte mutant pour *enok*.

L'objectif de ma thèse a été de comprendre les mécanismes par lesquels Enok régule la formation des CC chez la larve de Drosophile.

Les CC sont générées au stade larvaire à partir de la transdifférenciation de macrophages après activation par la voie Notch, et l'initiation de Lz. Au contraire de données publiées récemment par un autre groupe, j'ai démontré que Lz est requis et suffisant pour induire l'expression de l'effecteur de la voie Hippo Yorkie, et non l'inverse. De plus, grâce à des expériences de perte de fonction et de sauvetage phénotypique, j'ai montré qu'Enok est requis pour l'expression de Lz de façon autonome cellulaire dans les précurseurs de CC.

Chez les mammifères, MOZ est connu pour faire partie d'une tétrade d'acétylation, et j'ai montré qu'ici seul un de ses partenaires est requis, le facteur de type BRPF Br140. Pour approfondir l'étude de la fonction d'Enok, j'ai généré grâce au système CRISPR/Cas9 un allèle catalytiquement inactif d'*enok*. J'ai ainsi montré que cette fonction n'est absolument pas requise durant la différenciation des CC.

Enfin, j'ai montré qu'Enok permet la régulation de l'expression de Lz en se fixant sur un enhancer dans le troisième intron du gène. De façon intéressante, sa délétion provoque le même phénotype de perte de CC qu'une perte de fonction d'*enok*, montrant qu'il est requis pour l'expression de Lz dans le système hématopoïétique.

Ces résultats mettent en valeur un nouveau mode d'action d'Enok dans l'hématopoïèse chez la Drosophile. De façon intéressante, une étude récente propose qu'Enok et Br140 se fixent sur la chromatine pour réguler l'expression de gènes cibles. Mes résultats sur une fonction non-catalytique d'Enok viennent enrichir ce modèle, qui montre un mode d'action peu exploré dans le domaine. Dans le futur, ce travail pourrait ouvrir des portes sur une meilleure compréhension des fonctions normales et pathologiques de MOZ chez les mammifères.

Hematopoiesis is a very tightly regulated process leading to the normal production of every blood cells in an organism. In mammals there is a lot of cell types that participate to the establishment of the defense mechanisms of the body. All those cells come from the terminal differentiation of a single cell called Hematopoietic Stem Cell (HSC) which will differentiate to give rise to committed progenitors that will eventually differentiate all blood cell types. HSCs are among the most controlled cells in the organism. Indeed, deregulation of their normal function (excessive proliferation, premature differentiation, ...) can be at the onset of severe blood pathologies called leukemias.

Several molecular factors are involved in HSC regulation, and some of them, like RUNX1 and the Monocytic Leukemia Zinc-Finger protein (MOZ), are targets of mutations or chromosomal rearrangements that lead to a leukemic transformation.

In Drosophila, blood cells share functional homology with the mammalian myeloid lineage, and the molecular actors controlling their formation are well conserved. Indeed, differentiation of crystal cells (CC), which have similar functions than megakaryocytes, occurs following the interaction of RUNX1 homolog, Lozenge (Lz) and GATA1 Serpent (Srp). With the aim at identifying regulators of Srp/Lz transcriptional activity, a genome-wide screen led by my team allowed the identification of *enoki mushroom* (*enok*) as a strong negative regulator of this activity. Enok is the homolog of MOZ in Drosophila, and a preliminary study *in vivo* showed that Enok is essential for CC development during the larval stage, as CC almost completely disappear in *enok* loss of function context.

The objective of my PhD was to understand the mechanisms by which Enok regulates CC formation in the Drosophila larvae.

During the larval stage, CC are generated by the transdifferentiation of macrophages, after their activation by the Notch signaling pathway and onset of Lz expression. In contrast to data published by another group, I demonstrated that Lz is required and sufficient to initiate the expression of the Hippo signaling pathway effector Yorkie, and not the contrary. Furthermore, using loss of function and rescue experiments, I showed that Enok is absolutely required cell autonomously in CC precursors for Lz expression but not for the proper Notch signaling which appears normal in an *enok* mutant context.

In mammals, MOZ is known to be a part of an acetylation complex, and I showed that only one of its members, the BRPF factor Br140, is required alongside with Enok during CC differentiation. To go further into the characterization of Enok mode of action, I generated a catalytically inactive *enok* mutant using the CRISPR/Cas9 system and demonstrated that this function is completely dispensable for CC differentiation in the larvae.

Finally, I show that Enok regulates the maintenance of *Lz* expression by binding to an enhancer located in its third intron. Hence, deletion of this enhancer provokes the same loss of CC phenotype as what is observed after *enok* loss of function showing that this enhancer is required for *Lz* expression in the hematopoietic system.

These results highlight a new mode of action of Enok during Drosophila hematopoiesis. Interestingly, a recent study proposed that Enok and Br140 bind chromatin together in order to regulate gene expression. Therefore, my results on a non-catalytic activity of the drosophila Enok brings new insights into this new model, which uncovers a mode of action not much explored in the field. In the future, my work should open new avenues and might allow a better understanding of MOZ normal functions in mammals and thus give rise to more efficient ways to treat MOZ-associated pathologies.