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Review

Signal transduction pathways, intrinsic regulators, and the control of cell fate choice ☆

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ABSTRACT

Background: Information regarding changes in organismal status is transmitted to the stem cell regulatory machinery by a limited number of signal transduction pathways. Consequently, these pathways derive their functional specificity through interactions with stem cell intrinsic master regulators, notably transcription factors. Identifying the molecular underpinnings of these interactions is critical to understanding stem cell function.
Scope of review: This review focuses on studies in *Drosophila* that identify the gene regulatory basis for interactions between three different signal transduction pathways and an intrinsic master transcriptional regulator in the context of hematopoietic stem-like cell fate choice. Specifically, the interface between the GATA:FOG regulatory complex and the JAK/STAT, BMP, and Hedgehog pathways is examined.
Major conclusions: The GATA:FOG complex coordinates information transmitted by at least three different signal transduction pathways as a means to control stem-like cell fate choice. This illustrates emerging principles concerning regulation of stem cell function and describes a gene regulatory link between changes in organismal status and stem cell response.
General significance: The *Drosophila* model system offers a powerful approach to identify the molecular basis of how stem cells receive, interpret, and then respond to changes in organismal status. This article is part of a Special Issue entitled: Biochemistry of Stem Cells.

1. Introduction

Stem cells have the dual capacity to self-renew and differentiate, thereby replenishing the stem cell pool and producing the entire spectrum of cells that form a given tissue. These characteristics underlie the ability of stem cells to maintain tissue homeostasis throughout the life of an organism by replacing lost or damaged tissue and/or mounting a response to environmental assaults. This involves communicating changes in the status of the organism (organismal status), such as immune challenge, nutritional deprivation or wounding, to the often sequestered stem cell compartment [1–4]. Signal transduction pathways serve in this capacity by transmitting remote information to the stem cell regulatory machinery in order to maintain homeostasis and initiate the appropriate cellular response. Interestingly, this information is thought to be communicated by relatively few signal transduction pathways, which also function across different stem cell systems [5]. Additionally, within a given system, these pathways have been shown to promote both stem cell self-renewal and differentiation [6–9]. Signal transduction pathways appear to derive their functional specificity by interacting with intrinsic stem cell master regulators, notably transcription factors [5]. Additional levels of functional specificity are achieved by the convergence of multiple signal transduction pathways that interface with one or more intrinsic master regulators. Ultimately, this convergence alters the stem cell-specific gene regulatory landscape and thereby determines cell fate choice [5,10].

The blood organ is a dynamic system that produces a number of different cell types that respond to a wide range of changes, including immune challenge and aging [1–4,11–18]. Consequently, hematopoiesis is an excellent system to investigate how signal transduction pathways interface with specific master regulators to control cell fate choice. Although *Drosophila* has a rudimentary hematopoietic system, powerful genetics coupled with a short generation time makes this an ideal model to investigate the molecular basis for regulatory strategies that link these changes in organismal status with cell fate choice. Importantly, because these studies are conducted in vivo, the response to changing conditions is governed by the regulatory complexity imposed by the whole organism [19].

Recent studies in the fly suggest that the GATA transcription factor when bound to the co-regulator Friend of GATA (GATA:FOG complex) is a master regulator, which controls hematopoietic cell fate choice through interactions with the following three signal transduction pathways: 1) Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT); 2) Bone Morphogenic Protein (BMP); and 3) Hedgehog (Hh) [20–22]. Specifically, the GATA:FOG complex functions to maintain multilineage developmental potential (multipotency) and block differentiation of blood cell progenitors (stem-like cells). Importantly,

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changes in the relative levels of GATA to FOG also alter fate choice [21]. GATA:FOG complex formation is regulated by the JAK/STAT and BMP signal transduction pathways, which may be important mechanisms that mediate the response to changing environmental conditions [20,21]. Of equal importance are the downstream effectors of the GATA:FOG complex. GATA singularly, and when bound to FOG, regulates the Hedgehog expression domain [22]. Tight regulation of Hedgehog is required to maintain the stem-like cell population [23]. Collectively, these observations suggest that the GATA:FOG complex serves as a nexus that coordinates information carried by three different pathways to regulate cell fate choice. Overall, these findings may provide a conceptual framework for studies designed to investigate how information is received, interpreted, and acted upon by stem cell systems. This review presents the findings that identified the molecular basis for the interaction between these signal transduction pathways and the GATA:FOG complex. Additionally, a discussion of how these interactions may control cell fate choice during steady-state hematopoiesis and in response to immune challenge is presented.

2. *Drosophila* hematopoietic system

2.1. The Drosophila hemocytes and hematopoietic organ

Drosophila blood cell progenitors have been described as stem-like cells because they share key characteristics with mammalian hematopoetic stem cells (HSCs), including quiescence, multipotency, and niche-dependence [23–25]. *Drosophila* stem-like cells give rise to all three of the mature blood cell types: 1) plasmatocytes are operational macrophages that mediate phagocytosis of bacterial pathogens and apoptotic bodies; 2) crystal cells are named for their crystalline inclusion bodies, and are involved in wound healing; and 3) lamellocytes are normally rare blood cells that are produced in large numbers in response to various types of immune challenge [20,21,23,24,26–38].

Drosophila hematopoiesis takes place during two spatially and temporally distinct periods or waves, which is similar to the pattern seen in vertebrate blood systems. The first wave takes place in the embryonic head mesoderm, whereas the second wave takes place in a specialized organ known as the lymph gland [39]. An elegant study using lineage analyses of transplanted cells demonstrated that the blood cells of the head mesoderm and the cells of the primordial lymph gland arise from two different anlagen. Furthermore, this approach was instrumental in demonstrating that blood cells from both the first wave (head mesoderm) and second wave (lymph gland) persist throughout the adult stage of the fly [40].

During embryogenesis, the lymph gland is specified from the cardiogenic mesoderm and develops from hemangioblast-like cells that have the potential to become either heart (dorsal vessel) or blood cells. The embryonic lymph gland is a bilateral organ containing one pair of primary lobes that flank the heart [41]. The primary lobes contain two distinct cell types, comprising approximately 20 hematopoietic stem-like cells and a cluster of five or six non-hematopoietic cells that sit at the posterior base and give rise to the Posterior Signaling Center (PSC). The PSC functions as the stem cell niche [23,24,42].

In *Drosophila* development, the embryonic stage is followed by three larval instars. During the larval instars, the lymph gland cells proliferate, increasing in number by approximately 100-fold. By the early third larval instar, additional paired secondary lymph gland lobes have formed posterior to the primary lobes [35]. The lymph gland reaches full maturity by the middle of the third larval instar [24]. The primary lobes contain stem-like cells, precursors, and terminally differentiated blood cells [23,24,35,43]. At this stage, the primary lobe is organized into three regions or zones with distinct hematopoietic functions (Fig. 1). The first is the PSC or niche, which maintains stem-like cell quiescence and multipotency through several signaling pathways [23,24,44,45]. The second or medullary zone contains the stem-like cells. During the process of differentiation, these cells migrate to the third region called the cortical zone. Here, they continue to develop and give rise to all three blood cell types [23,24,35,44].

2.2. The Drosophila stem-like cells

Mammalian HSCs are characterized using functional assays that assess the capacity to continuously regenerate all blood cell types. This involves transplantation of heterogeneous populations of cells into irradiated animals and assaying for repopulation of all the blood lineages [46,47]. This method is considered the gold standard for identifying HSCs. Repopulation assays are currently not feasible for studies using *Drosophila*. Instead, investigators rely on lineage tracing studies and the persistence of marked clones to identify putative stem cell populations [48,49]. Using this approach, one study provided evidence for *Drosophila* HSCs within the embryo and the first larval instar lymph gland. However, it was not possible to definitively identify HSCs in the second and third larval instar using this approach. The interval between clone induction in the second larval instar and analyses in the third larval instar is too short to distinguish between transient clones representing dividing precursors and persistent clones representing self-renewing stem cells [49]. In another study using this method, the authors concluded that all lymph gland cells become committed precursors by the end of the first larval instar [48]. However, heat shock was used to induce clones in both of these studies [48,49]. Heat shock changes the gene expression landscape, which could alter progenitor cell potential and may account for some of the differences between these two studies. On the other hand, lineage tracing studies performed without the use of heat shock showed that second larval instar progenitors can give rise to all the mature blood cell types [35]. This suggests that when the lymph gland reaches maturity in the mid-third instar, the medullary zone contains a heterogeneous population of cells, which most likely ranges from bona fide stem cells to more advanced progenitors. Indeed, a recent study demonstrated for the first time the heterogeneity of the medullary zone population. This study showed that two distinct markers, Domeless-Gal4 (Dome) and ZCL2897 (ZCL), are differentially expressed within this population. Three different cell

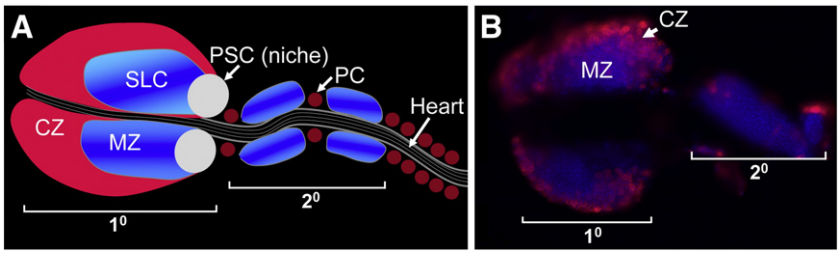

Fig. 1. The *Drosophila* hematopoietic lymph gland. (A) Schematic of the mature third larval instar lymph gland showing primary (1^0) and secondary (2^0) lobes. The relative positions of the three domains within the primary lobe are shown, specifically cortical zone (CZ), medullary zone (MZ), and stem cell niche (PSC; Posterior Signaling Center). Stem-like cells (SLC) reside in the MZ. Pericardial nephrocytes (PC) are insect renal cells that filter the blood and reside in two rows that flank the insect heart. (B) Lymph gland showing plasmatocytes in the CZ stained with the specific marker P1 (red). The medial region of the lymph gland contains densely packed, unstained MZ cells. The lymph gland is counterstained with Dapi (blue) and the 1^0 and 2^0 lobes are marked.