Regulators of Hematopoietic Progenitor Differentiation

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Abstract

Hematopoietic stem cells (HSCs) must be capable of both self-renewal and production of progenitor cells that give rise to all of the blood cell types, the two basic principles of stem cell biology. Investigations into gene networks controlling HSC response to stress have been conducted using model organisms such as *Drosophila melanogaster.* One of the factors controlling vertebrate HSC multipotency and differentiation is the GATA:FOG complex. GATA:FOG complexes regulate the development of a number of blood lineages. In *Drosophila*, the GATA:FOG complex blocks the differentiation of hematopoietic progenitors into the stress-responsive lamellocyte lineage. *Drosophila* hematopoietic progenitors are strikingly similar to HSCs and are used as a model for these vertebrate stem cells. We used fly genetics to identify GATA:FOG network members that block the stress-response of hematopoietic progenitors.

Introduction

Investigating the Hematopoietic Stem Cell Response to Stress

Investigating the molecular basis of the stem cell differentiation in response to stress will further our understanding of the regenerative capacity of these cells. Investigations into gene networks regulating differentiation have been conducted using in vitro methods, but can also be conducted using model organisms such as *Drosophila melanogaster. Drosophila's* hematopoietic system is ideally suited to rapidly identify gene regulatory pathways controlling cell fate choice during steady-state conditions and in response to stress. Similarities between HSCs and *Drosophila* hematopoietic progenitors are multipotency, quiescence and niche dependence.

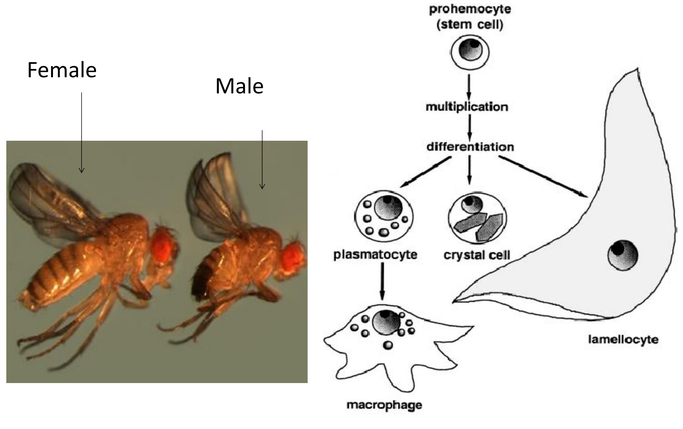


Figure 1. *Drosophila melanogaster* model systems. The system is simple compared to the vertebrate system, but the basic pathway with stem cells giving rise to terminally differentiated blood cells is similar. The three blood lineages in *Drosophila* are plasmatocytes, the most abundant cell type, which are operational macrophages, crystal cells, which function during wound healing, and lamellocytes, the rarest blood cell type under normal conditions, increases in number in response to immune challenge.

Introduction (cont.)

GATA:FOG complex Controls Hematopoiesis

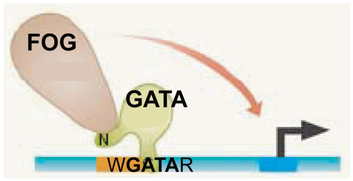
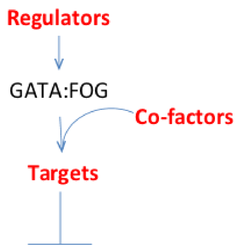


Figure 2. GATA factors are evolutionary conserved master regulators of hematopoiesis and organogenesis. Friend of GATA (FOG) binds and regulates GATA activated transcription. The *Drosophila* Hematopoietic GATA:FOG Complex is Srp:Ush. Serpent (Srp) is analogous to the three vertebrate GATA Factors. Ushaped (Ush) is *Drosophila* homologue of FOG.

Hypothesis

Identification of GATA:FOG factors that will act with the complex to block lamellocyte differentiation will help increase our understanding of how this complex limits progenitor differentiation and promotes multipotency.



Experimental Design

Genetic Screen of *Drosophila melanogaster*

•We conducted a genetic screen, based on the principle of second site-non-complementation, to detect production of stress-responsive lamellocytes and identify key factors that interface with this complex to limit the progenitor response to stress.

•Second Site Non-Complementation- when two different genes are recessive for a particular phenotype, loss of one functional copy of each gene produces a mutant phenotype.

Scoring

•Larval progeny was analyzed for presence of the m-Cherry (MSN-C) reporter gene, which is active in "muscles" and which is a marker for lamellocytes and presence of lamellocytes under fluorescent microscopy.

•The degree of lamellocyte differentiation, ranging from weak to strong, was determined.

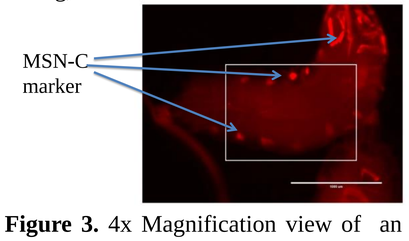


Figure 3. 4x Magnification view of an entire Larvae. The box delineates the region scored in the 10x magnification of the larvae. MSN-C marker is shown in the muscles, but not in lamellocytes as they are normally rare blood cell types.

Experimental Design (cont.)

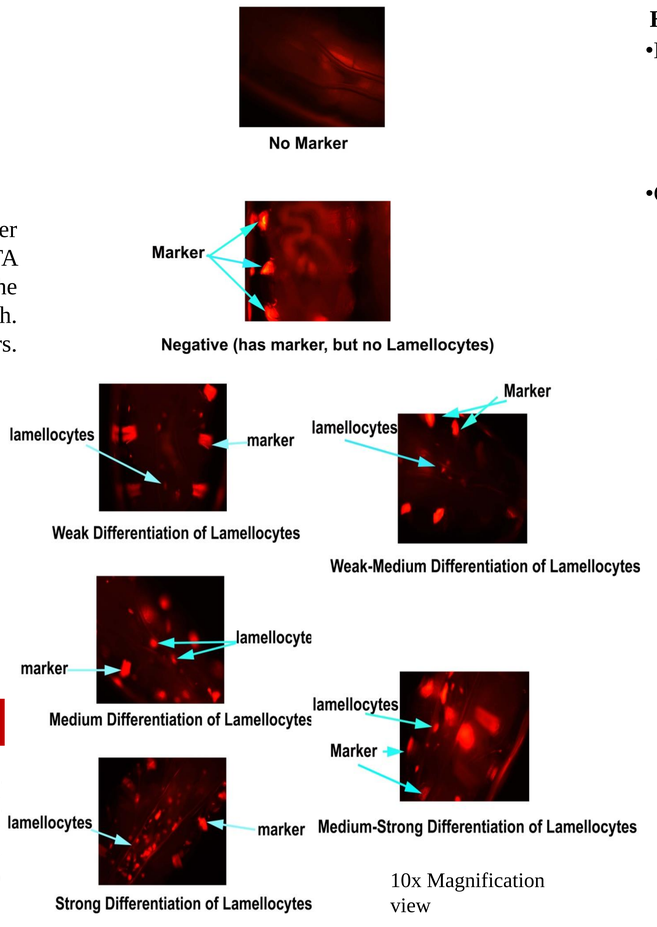


Figure 4. Larva with lamellocyte differentiation ranging from weak to strong. In the genetic screen a medium-strong to strong differentiation constituted to a positive result.

Results

Table 1: Conserved factors that interact with Ush

Mammalian homolog	Stress related function
Thioredoxin	Component of glutathione redox system
Eyes absent tyrosine kinase	Phosphatase: regulates immunity
E2F2	Cell cycle and tumor suppression
VDAC	Mitochondrial membrane protein: apoptosis
Sec61α	Protein translocation, endoplasmic reticulum
Intersectin	Regulation of Ras activation of MAPK kinases
Glyoxylate reductase	NADP binding oxidation-reduction

Drosophila homologs of genes listed in Table 1 were identified as acting with Ush to block lamellocyte differentiation. These genes include Cl^{1} $eya^{cli\text{-}IID}$, $E2f2^{76Q1}$, $porin^{k05123}$, $Sec61\alpha^{k04917}$, $Dap160^{\Delta 2}$, $CG31673^{KG01187}$

Future Work

Hypotheses of Future Experiments

•Knockdown of these genes will result in an
- •Increase of differentiated cells in the lymph gland.
- •Decreased progenitor cells due to increased differentiation

•Candidate genes may function as
- •Downstream targets of Ush
- •Regulators of Ush
- •Cofactors with Ush

Knockdown of Candidate Gene Expression in the *Drosophila* lymph gland with RNAi Using the UAS/GAL4 System.

Expression of each candidate gene will be knocked down in the lymph gland of *Drosophila* through RNAi. The RNAi will be performed using the UAS/GAL4 system.

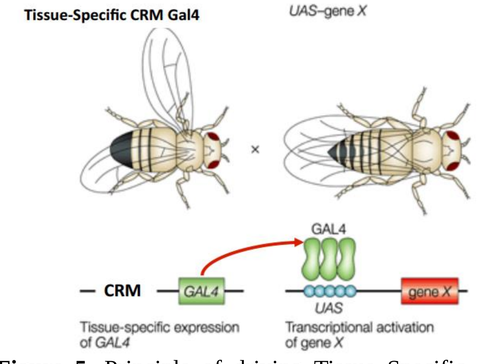


Figure 5. Principle of driving Tissue Specific gene expression through the UAS/Gal4 system. In the resulting progeny, Gal4 will bind to the UAS, which will result in activation and expression of the gene of interest in the progeny. The gene to be expressed in our study is siRNA of each candidate gene, while the specific tissue will be lymph gland tissue.

Conclusions

•Second site non-complementation screen can be used to identify factors that act with the GATA:FOG complex in the fly.

•We hope to gain further knowledge of the molecular basis for the stem cell response to stress.

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