Gonadogenesis and germ line stem cell establishment

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Overview

A major question in developmental biology is how do organs form and function. A particular aspect of this question: the employment of stem cells in organ formation and function is the focus of intensive research. Studying how stem cells participate in organogenesis, homeostasis and regeneration is crucial not only to our understanding of normal body function, but also to our understanding of disease, for designing cures and for regenerative medicine. The major goal in our lab is to study gonad development in the fruit fly *Drosophila melanogaster* as a model for organogenesis and stem cell establishment.

A large part of gonadogenesis in Drosophila females occurs during the larval stage of the fly's life cycle. Events such as proliferation, differentiation, morphogenesis of the somatic niche and the establishment of Germ Line Stem Cells (GSCs) all happen in the larva (figure 1). Our lab focuses on three major aspects of organogenesis: growth, differentiation and stem cell establishment. Specifically, we study how the somatic niche for germ line stem cells is formed, how niche formation affects the establishment of GSCs from their precursor cells, and how the growth of several cell lineages is coordinated in the larval ovary.

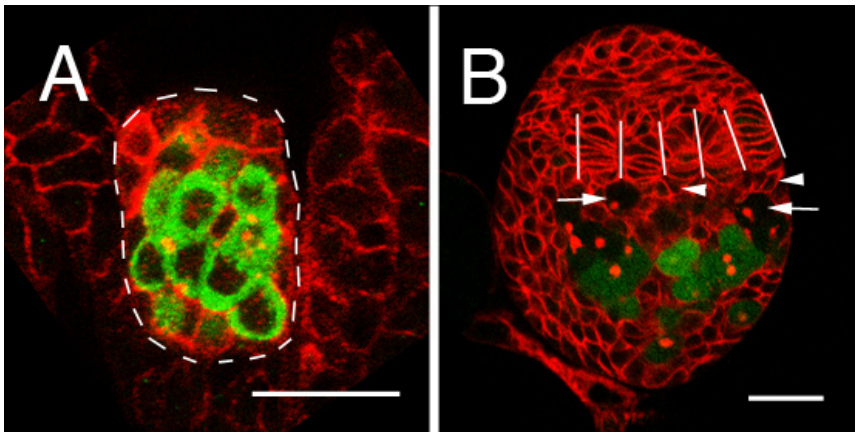

Fig. 1 *Larval ovary development (A) At the end of embryogenesis, the ovary is small and displays little differentiations. Somatic cell membranes are labeled red, germ cells are green. (B) By the end of larval development, somatic niches for germ line stem cells are formed (terminal filament, between white lines, and cap cells, marked by arrowheads). Juxtaposed to the somatic niches are the germ line stem cells (arrows). Red staining marks somatic cell membrane and the fusome, an organelle within germ cells. Green marks germ cells that are not maintained as stem cells, and differentiate to form the first egg chambers of the female. Bars are 20 µm.*

Niche formation and stem cell establishment

One question in the field of stem cell biology is what are the qualities of adult stem cells that differentiate them from other precursor cells. In Drosophila, Primordial germ cells (PGCs), the precursor cells for adult germ line stem cells (GSCs), divide symmetrically within the larval ovary; each division produces two PGCs. Once the somatic niches for GSCs differentiate, PGCs become GSCs, which divide asymmetrically to produce one GSC and a differentiating daughter cell. We have previously shown that, unexpectedly, both PGCs and GSCs share an important feature of 'stemness': repression of differentiation. Thus, mechanisms and molecules that repress GSC differentiation, function identically in repressing PGC differentiation. The newly formed niche does induce asymmetry in GSCs, which is not present in the precursor cells, and might induce other, as-yet unknown, changes.

To define the changes within precursor cells as they become stem cells, we are using a microarray analysis comparing PGCs to GSCs. PGCs and GSCs share much of their transcriptional program. Thus, the differences should be limited to those induced by the changing niche. We will focus on transcripts that change as PGCs become GSCs, and that encode secreted or trans-membrane proteins, which might indicate a change in communications between germ cells and the newly formed niche. Transcripts that might suggest a difference in asymmetric localization of proteins, chromatin status or cell cycle regulation will also be of interest. Many mutants and RNAi flies are available to facilitate an analysis of the candidates' biological function.

A screen for new genes affecting stem cell maintenance and gonad morphogenesis

Numerous screens for genes that affect female fly fertility have been carried out. Despite these numerous unbiased genetic screens, most genes that are known to play a role in GSC maintenance and differentiation were found using a candidate gene approach. In addition, genetic screens have largely failed so far to uncover genes that are important for gonad morphogenesis.

To circumvent the problems associated with with traditional screens, we performed a mis-expression screen assaying for morophogenesis defects and precocious PGC differentiation directly in the larval ovary (unpublished).

The screen has yielded genes that were previously discovered by a candidate gene approach. It also uncovered many novel candidates and phenotypes (Figure 2). We will be using genetic manipulations and molecular

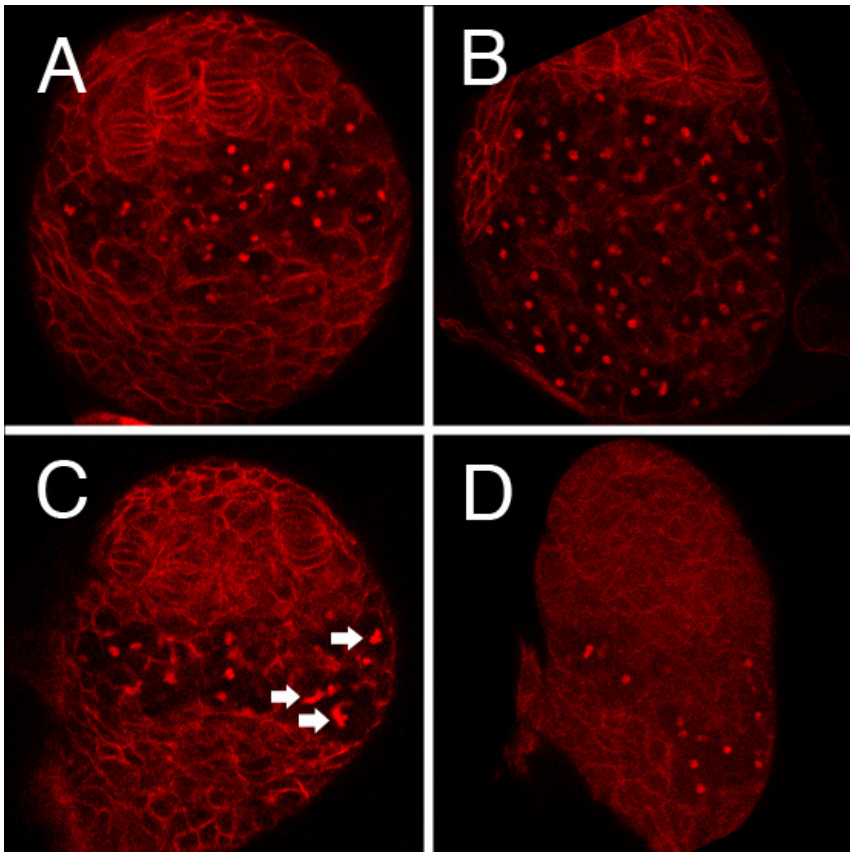

Fig. 2 *sample of screen phenotypes. In all images, red staining marks somatic cell membranes and the fusome, an intracellular organelle within germ cells that is round at larval stages. (A) wild type ovary, terminal filament and round fusomes within germ cells are apparent. (B) Over proliferation of germ cells within the larval ovary as revealed by too many round fusomes. (C) Extended fusome morphology (arrowheads) denotes precocious differentiation of PGCs, suggesting stem cell maintenance defects. (D) Severe morphogenesis defects of the ovary. Terminal filament, the niche for germ line stem cells, fail to form.*

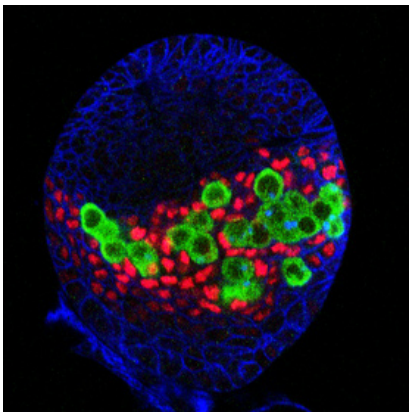

Fig. 3 *Close proximity of ICs and PGCs. Germ cells (green) and Intermingled cells (red) are closely associated and reside at the middle of the ovary at late larval stages. Somatic cells membranes are labeled blue.*

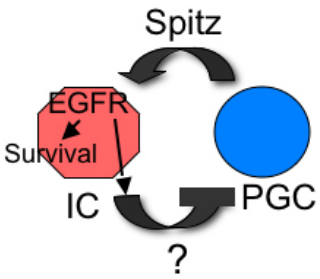

Fig. 4 *A homeostatic feedback loop between ICs and PGCs See text for explanations.*

markers to test the function of the candidates in gonad formation and stem cell function.

Coordinated growth of an organ

Work in many labs established that organ size is affected by extrinsic mechanisms, such as nutrient availability and hormonal regulation. It is also controlled by organ-intrinsic mechanisms such as growth factors and adhesion molecules. Our lab is interested in a much less understood problem in organogenesis: how different cell types within the organ coordinate their growth, such that there is no lack or excess of any cell type. In more general terms, we are trying to understand how the differentiation of the various cell types within the ovary intersects with growth control mechanisms.

We have recently shown that primordial germ cells (PGCs) and a somatic group of cells termed Intermingled cells (ICs) coordinate their growth during larval gonad formation (Figure 3). PGCs produce Spitz, a ligand for the Epidermal Growth Factor Receptor (EGFR), which is required for ICs survival. In return, ICs produce an unknown factor that represses PGC proliferation (Figure 4). The feedback loop coordinates proliferation and survival of two cell types. It also has homeostatic properties. Such feedback loops might also underlie homeostatic processes and organs in vertebrates.

We are now establishing how the feedback loop between PGCs and ICs is integrated with other growth signals within the ovary. We are especially interested in the role Decapentaplegic (Dpp, a BMP2/4 homologue) might play in coordinating somatic differentiation with PGC proliferation. Dpp is expressed in the larval ovary and promotes PGC proliferation. We assume that ICs repress PGC proliferation by interfering with PGCs receiving the Dpp signal. Dpp is also required for somatic cell proliferation and perhaps differentiation. We hypothesize that Dpp is an organizer of the ovary and as such coordinates the growth and fate of many cell lineages.

Selected publications

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INTERNAL support

Helen and Martin Kimmel Stem Cell Center