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Meeting Report

# Current topics in organogenesis and gametogenesis

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**At the 49th Annual Drosophila Research Conference from April 3–8, 2008 in San Diego there were eight talks and over ninety posters in the section on Organogenesis and Gametogenesis. These covered a wide range of topics within the two broad categories of organ-specific stem cells (including germ cells) and organ-specific developmental programs. Here we discuss eleven of these presentations describing current research into the formation of the gonad, intestine, trachea, muscle and leg joint. The new insights presented advance our understanding of the molecular events that underlie interactions between stem cells and their niches as well as mechanisms underlying tissue-specific differentiation programs.**

## Stem Cells

**Gonad.** The primary factor controlling sexual dimorphism in flies is the transcription factor Doublesex (Dsx), which has a male specific and a female specific form, with each apparently promoting the properties characteristic of one sex and repressing those of the other. The different isoforms are generated by differential splicing that is dependent upon the ratio of X chromosomes to autosomes.[1] Although it is generally assumed that this is a cell autonomous process, there is evidence that sexual dimorphism is controlled cell non-autonomously in some tissues and more recently that Dsx is expressed only in a subset of cells. This was supported by a recent study from the Van Doren lab (Johns Hopkins University) investigating how the pigment cells, which surround the testis, are specified.[2] These are male specific cells and there is no counterpart in female gonads. This cell type is induced non-autonomously by fat body mesoderm in male gonads during embryogenesis, the inducing factor being Wnt2. In addition, it was shown that Dsx is not required for the development of this cell type, suggesting only the female-specific form is required in females to repress their development. Recently, work from Leonie Hempel and Brian Oliver has shown that Dsx is not expressed in the pigment cells,[3] further supporting the conclusion that the sex determination pathway acts non-autonomously to control male-specific development of these cells. The presentation by Nicole Camara from the Van Doren lab next turned to the development of the hub cells, which are male specific cells that form the germline stem cell niche. Hub cells express Dsx (reviewed in ref. 3) and undergo autonomous sex determination. Nevertheless, in *dsx* mutant embryos the hub cells still develop, suggesting that male is the default state at least initially. However, if these are followed through to the third instar only about half of the mutants retain a hub, the remaining ones possess a terminal filament characteristic of female gonads. Interestingly, both XX and XY *dsx* mutants show a similar distribution of hubs vs. terminal filaments. Thus, it appears that although development initially proceeds along a male pathway the gonad subsequently seems to make a stochastic decision to continue down this pathway or to change direction into a female. Therefore, initially *dsx* is required primarily to repress male gonad development in females, while later it tips the balance between maintenance of the male gonad vs. development of the female gonad.

Sexual specification of the germ cells is regulated by a combination of autonomous cues, that are independent of Dsx, and signals from surrounding somatic cells of the gonad, one of which is the Jak/Stat ligand, Unpaired.[4] Abbie Casper, also from the Van Doren lab, presented a study with the goal of uncovering genes that control the normal behavior and differentiation of the germ cells. They have identified several genes that are activated specifically in the male germ line, including *no child left behind* (*nclb*; CG6751). *nclb* encodes a chromatin-associated protein that is required for maintenance of the male germline stem cells, and their ability to properly differentiate into sperm. Christopher Cherry from Erika Matunis's lab (John Hopkins University) also reported the importance of chromatin regulation in stem cell development in male gonads, here the NURF301 protein in stem cell maintenance. This protein is a component of a chromatin remodeling complex and has previously been shown to be required for Ecdysteroid signaling and metamorphosis in flies.[5] Clonal analysis revealed that NURF301 is required cell autonomously for maintenance of both germ line and somatic stem cells in testes.

Emerging evidence suggests that chromatin organization also plays a pivotal role outside the germline in establishing stem cell identity and in the maintenance of "stemness" in these cells over time. In stem cells, chromatin status (one measure of which is accessibility to transcription factors) is modulated such that the transcription of differentiation factors is prevented. However, the silencing of differentiation genes needs to be reversed in stem cell daughters destined to activate a particular developmental program. To gain insight into the regulation of chromatin status in stem cells and their daughters Mike Buszczak (then in Allan Spradling's lab; ref. 6)

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screened through a collection of GFP protein trap lines looking for germline stem cell enriched nuclear factors. These efforts led Mike (now a PI at UT Southwestern Medical Center) to focus his efforts on a ubiquitin specific protease referred to as Scrawny (Scny). In his poster, he showed that a wide variety of stem cells in both females and males express high levels of Scny in their nuclei and that the highest levels of Scny are found in the nucleolus. Interestingly, *scny* mutants are missing a diverse set of stem cell populations, including germline stem cells of the ovary and testis, follicle stem cells of the ovary and intestinal stem cells of the posterior midgut. Sequence similarity with two yeast proteins and direct evidence from biochemical experiments demonstrated that Scny deubiquitylates histone H2B. Following up, a genetic analysis suggested that *scny* functions in gene silencing in stem cells. While preliminary, this data suggest that modulation of histone H2B mono-ubiquitylation is among the regulatory switches employed to balance stem cell maintenance with stem cell daughter differentiation.

**Intestine.** Great excitement was generated by two recent reports[7,8] describing the identification of intestinal stem cells in the midgut endoderm of adult flies and the potential involvement of the Notch pathway in proliferation-differentiation decisions by these cells. This discovery provided a powerful experimental platform from which to dissect the molecular mechanisms underlying proliferation-differentiation decisions and also the choice between two distinct differentiation programs activated in daughter cells (enterocyte or enteroendocrine). Allison Bardin from Francois Schweisguth's laboratory (recently relocated to the Institute Pasteur) discussed a sophisticated genetic analysis of Notch pathway components in intestinal stem cell regulation. The highlight of the talk, in my opinion, was the employment of "double" MARCM clones in which a second clone is induced within the first clone. This system was employed to manipulate positive (e.g., Su(H) and E(spl)) and negative (e.g., H) pathway components in various combinations with Notch (enterocyte) and Delta (intestinal stem cell) expression as a readout. Their results suggest that the level of Notch signalling in stem cell daughters dictates the choice between self-renewal (loss of Notch) and activation of the enterocyte differentiation program (excess Notch).

**Trachea.** During metamorphosis the tissues of the adult fly are largely reconstructed from undifferentiated imaginal cells set-aside during embryogenesis and which can be found in various locations in the larva. This includes the tracheal system where a population of 'tracheoblasts' have previously been identified, associated with the spiracular branch of the larval tracheal system and were thought to be solely responsible for remodeling the system during metamorphosis. However, Molly Weaver from Mark Krasnow's lab (Stanford University) has identified a second population of cells in the dorsal branch of the larval system that are required for constructing part of the pupal system, and remarkably these are not undifferentiated imaginal cells (they do not express the diagnostic marker *escargot*), but are larval cells that form part of the larval tracheal system. Their behavior was followed in live animals with a GFP marker and they were shown to reenter the cell cycle and differentiate into three different cell types found in the pupal tracheal system; this differentiation being influenced by FGF signaling. This may be the first example of fly larval cells that reenter mitosis during metamorphosis. A similar finding has also recently been reported by the Tabata lab.[9] These studies may provide insights into how presumably terminally differentiated cells can dedifferentiate to regenerate tissue following wounding or disease.

How flies generate the tubes of the tracheal system was the subject of a forward, mosaic genetic screen reported by Amin Ghabrial (University of Pennsylvania); the screen was carried out in collaboration with Boaz Levi in Mark Krasnow's lab. During larval life new trachea are formed at the termini of preexisting ones by the cells in these locations sending out thin cellular extensions within which a lumen forms converting the extension into a tube.[10] Mutations in around seventy genes were uncovered that disrupt this process and here the forty or so mutants that affected the formation of functional seamless tubes were reported. These could be divided into two groups. In the first, formation of the tube was defective so that a lumen may be completely lacking, or it may form and have an irregular diameter or it may develop into a group of separate isolated vacuoles rather than a continuous tube. Mutants in the second group formed tubes but these were not cleared and fail to fill with air. Lumen formation is thought to involve fusion of vesicles so it was encouraging to find that some of the mutations encode proteins involved in this process, including *whacked* encoding a Rab GAP and *moon cheese*, encoding a t-SNARE. Mutants identified in the second group included some already known to be involved in tracheal development, *knickkopf* and *krotzkopf verkehrt*. As well as uncovering new proteins involved in tracheal morphogenesis, these studies may provide novel insights into how tubes form during development of different vertebrate tissues.

## Development

**Intestine.** The endoderm is derived from two distinct primordia located near the poles of the embryo that migrate toward each other and fuse midway through embryonic development. This is quite different from the origins of the ectoderm and mesoderm—from the earliest stages of development these tissues extend the full length of the embryo and individual cells contain anterior-posterior information within them. Studies over many years have shown that endoderm cells receive anterior-posterior cues, in the form of secreted proteins, from the overlying mesoderm. One well-studied example is the Dpp signal that emanates from parasegment 7 of the visceral mesoderm to induce the expression of the Hox gene *labial* in the underlying endoderm (reviewed in ref. 11). Labial expression is required for differentiation of these cells into copper cells, a cell type with numerous micro-villi specialized for nutrient absorption.[12] Sergio Casas-Tinto in Pedro Fernandez-Funez' lab (UT Medical Branch Galveston) shed new light on endoderm differentiation by implicating the FoxK protein in Labial activation. The first part of the talk focused on the initial characterization of the FoxK transcription factor including: (1) a demonstration that it binds DNA and induces transcription in S2 cells; (2) that its RNA and protein are present in parasegment3 and parasegment7 of the midgut endoderm; and (3) that excision mutants do not display any midgut constrictions. The mutant phenotypes in particular suggested a role in Dpp signaling. The second part of the talk focused on a molecular genetic analysis designed to identify the role of FoxK in Dpp-mediated midgut endoderm differentiation. This study demonstrated that midgut endoderm differentiation is achieved through the sequential activation of transcription factors: first Dpp signaling from the mesoderm

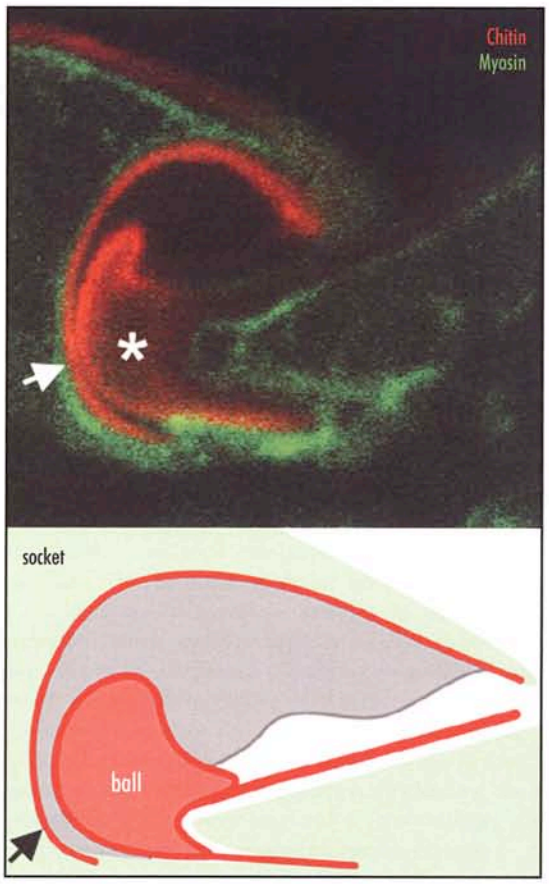


Figure 1. Development of the ball and socket joint between each tarsal segment of the leg.

activates Mad and Medea in the underlying endoderm, these Smads then directly activate FoxK and Dfos; these two proteins, in turn, activate Labial expression.

**Muscle.** How each of the 30 distinct body wall muscles of the trunk hemisegments is properly located is a longstanding question in fly organogenesis. Further complicating this topic is the necessity of explaining how intra-segmental or dorsal-ventral differences in muscle identity are incorporated into the muscle pattern in a position-dependent manner. Two talks addressed this question from different perspectives. A talk by Jonathan Enriquez from Alain Vincent's lab (Center for Developmental Biology, Toulouse) focused on how early pan-mesodermal patterning events are linked to later muscle-specific differentiation programs along the anterior-posterior axis. The model system described in the talk employed *collier* expression as both a determinant and read-out of DA3 muscle identity. The first part of the talk described studies characterizing the *collier* upstream region that revealed the existence of two phases of cis-regulation. The first phase correlated with conserved binding sites for the mesodermal transcription factor Nautilus and for *collier* autoregulation. This phase of regulation was shown to propagate "DA3 muscle identity" to all Fusion-Competent Myoblast nuclei that contribute to the DA3 myofiber as reported by this group recently.[13] The second part of the talk focused on the second phase of cis-regulation, one mediated via a new DA3 promuscular cis-regulatory module upstream of *collier*. Although this cis-regulatory module is active in all thoracic and abdominal segments, the DA3 muscle does not form in T1, indicating a key role of homeotic information at the progenitor/founder cell stage in implementing the muscle differentiation program. Current efforts are focused on determining if the homeotic gene *Antennapedia* interacts with *collier* in the late phase of DA3 muscle formation in T1.

A talk by Cheng Zhang from Markus Noll's lab (Institute for Molecular Biology, Zurich) focused on how early pan-mesodermal patterning events are linked to later muscle-specific differentiation programs along the dorsal-ventral axis. The model system employed *Pox meso* (*Poxm*), a transcription factor of the Pax1/9 family, as a central player in the formation of somatic muscles. The first part of the talk described a phenotypic analysis of *Poxm* mutants and genetic analysis of *Poxm* expression in wild-type and mutant backgrounds. This study revealed, as shown above for *collier*, that there are two distinct functions for *Poxm* in myogenesis. An early function, stimulated by Twist, is essential for designating a domain of competence for ventral and lateral muscle differentiation. The second part of the talk focused on a late phase of Poxm function in which it is required for specification of DT1 and VA1-3. Noll's group recently reported both functions for Poxm.[14] Current efforts are devoted to analyzing a cis-regulatory region of *Poxm* activated solely during its late phase of activity.

**Leg joints.** The legs of flies are flexible because of the specialized joints between adjacent segments. Studies on the mechanisms involved in establishing where these joints form in the developing leg imaginal discs have revealed that localized Notch signaling is key to their positioning, while later a localized boundary of Dpp signaling is required to induce localized cell death involved in tissue folding at the site of the joint.[15-18] However, the cellular mechanisms involved in actually constructing a joint are much less well understood, in particular for the ball-and-socket type joints that form between the tarsal segments. These are analogous to our own hip joints but in flies the ball and the socket are constructed from cuticle secreted from the epidermal cells forming the joint. Work from Reiko Tajiri in Shigeo Hayahi's lab (RIKEN Center for Developmental Biology, Kobe) has revealed that the cuticle making up the both the ball and the socket is secreted by the same cells on the proximal side of the joint: the ball is produced first, which then detaches, followed by secretion of cuticle on the dorsal/proximal side to form the socket (Fig. 1). This process obviously requires a clear orchestration of the behavior and cuticle secreting activity of the joint cells in molding ball and then the socket. Further analysis revealed that these events also require active Notch signaling, here functioning later than the initial activity required for positioning the joints.

## Summary

These studies, and others not mentioned here due to space constraints, showcase the power of genetic analysis to illuminate the complex processes of organ and gamete formation. New discoveries are clearly on the horizon that will reveal more information about the relevant molecular mechanisms. For those mechanisms that are conserved in cognate vertebrate tissues, discoveries in flies could suggest new experimental directions for investigators in the burgeoning field of regenerative medicine.

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