Drosophila oogenesis: **Coordinating germ line and soma**

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A new function for Delta–Notch signaling has been discovered in *Drosophila* oogenesis: Delta expressed in the germ cells activates Notch in the surrounding somatic follicle cells to control their differentiation, proliferation and morphogenesis.

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The *Drosophila* egg chamber is composed of two cell types, germ line cells and somatic follicle cells. The establishment of the body axes of the fly depends on reciprocal interactions between these cell types. An early indication that such interactions are important for anterior-posterior axis formation came from the analysis of *Notch* mutant egg chambers [1]. Since then, many papers have been published on the role of *Notch* in oogenesis, but its precise function remained elusive. Two recent papers [2,3] have considerably clarified our understanding of the processes in which *Notch* is involved, and moreover one of them [3] describes a new role for *Notch* in controlling cell proliferation and differentiation.

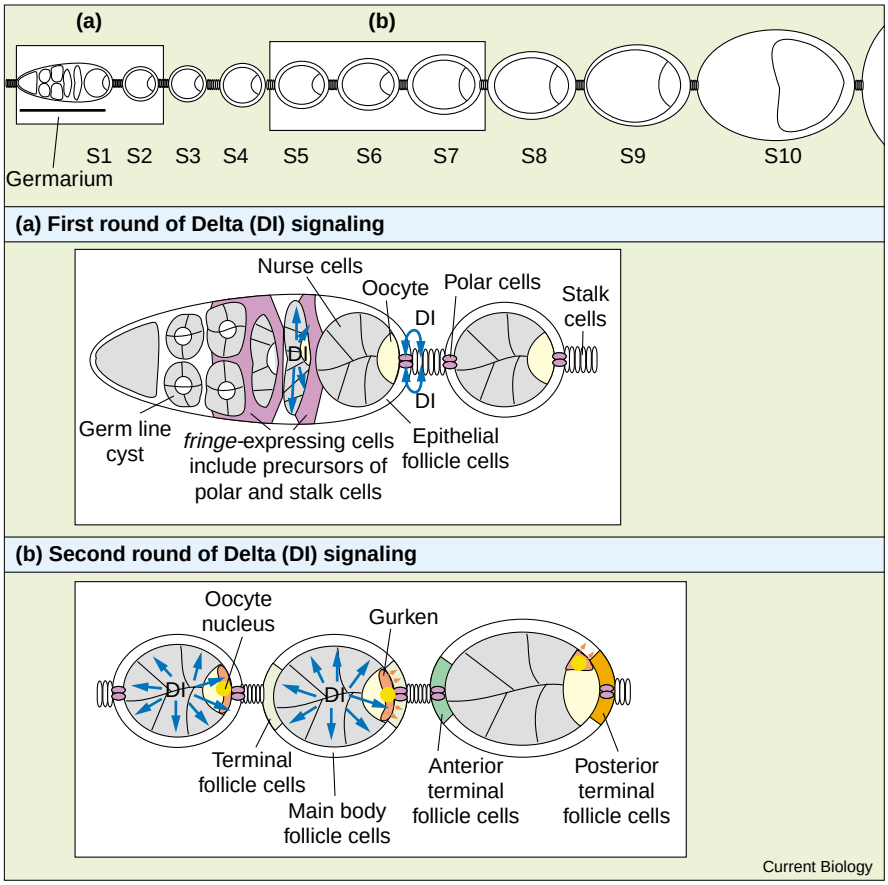
The *Drosophila* egg chamber forms at the anterior end of the ovary in a structure called the germarium (Figure 1) which harbours the germ line and the somatic stem cells [4]. In the germarium, the germ line stem cells divide asymmetrically and give rise to cystoblasts. A cystoblast undergoes four rounds of incomplete divisions to produce a cyst of 16 interconnected cells. Cysts are surrounded by somatically derived follicle cells through a complex morpho-morphogenetic process in which follicle cells migrate in-between the cysts. They cover individual cysts by an epithelial cell layer and separate the encapsulated cysts through the formation of stalks of six to eight cells. This process generates a chain of individualised egg chambers of progressive age (Figure 1).

Only one cell within a cyst will adopt the oocyte fate, while the remaining 15 develop into nurse cells which provide the synthetically quiescent oocyte with RNAs and proteins. The oocyte attaches through E-cadherin-mediated adhesion to follicle cells at the posterior end of the emerging egg chamber [5]. This confers an intrinsic anterior-posterior polarity to the egg chamber, with the nurse cells being anterior and the oocyte posterior. The posteriorly localised oocyte produces the TGF-α-like ligand, Gurken, which is secreted and activates the *Drosophila* EGF receptor (DER) in the abutting follicle cells. These cells adopt posterior fates upon DER activation. Subsequently, the posterior follicle cells signal back to the oocyte to polarise the oocyte cytoskeleton. This directs the localisation of *bicoid* and *oskar* mRNAs to anterior and posterior poles of the oocyte, respectively, and thus defines the anterior-posterior body axis of the later embryo. It also initiates the movement of the oocyte nucleus, which assumes an asymmetric position defining the future dorsal side of the egg chamber. Gurken becomes localised to the oocyte nucleus and a second round of Gurken signaling specifies dorsal follicle cell fates [5] (Figure 1).

Earlier work had shown that Notch is required for at least two important aspects of egg chamber formation [1,6–11]: first, for encapsulation of the germ line cysts by somatic follicle cells in the germarium; and second, for anterior-posterior polarisation of the oocyte. Early loss of Notch or its ligand Delta leads to formation of giant compound egg chambers, in which multiple germ line cysts are surrounded by a single follicular epithelium. Essentially, egg chamber formation is blocked. As this phenotype is characterised by the absence of interfollicular stalks, and as overexpression of *Notch* or *Delta* leads to formation of abnormally long stalks, Notch was suggested to determine the stalk cell fate [9]. Late loss of Notch and Delta causes mislocalisation of *bicoid* and *oskar* mRNAs; this is associated with fate changes in the posterior follicle cells required to signal back to the oocyte. Thus, Notch was suggested to control patterning and specification of the posterior follicle cells [1,9–11]. All of these studies, however, used weak or conditional alleles and did not determine in which cells Notch and its ligand(s) were actually required to fulfil their function.

The role of *Notch* in the early process of cyst encapsulation has now been clarified through careful analysis of cell clones mutant for *fringe*, *Notch* or *Delta* [2,3]. The *fringe* gene encodes a glycosyltransferase which modifies the Notch receptor and thereby modulates its responsiveness towards ligand activation [12–14]. For example, during wing development Fringe potentiates the ability of Notch to respond to its ligand Delta [15]. Interestingly, *fringe* is expressed in the germarium, in follicle cells which migrate between and separate adjacent cysts; later its expression gets restricted to the polar cells (Figure 1) [2,16,17]. The polar cells are pairs of rounded cells at each end of an egg chamber (Figure 1). They are the points of attachment of the stalk cells. Mutant clones reveal a surprisingly localised requirement of *fringe* function only in polar cells. Mutant *fringe* cells cannot adopt the polar cell fate, and the same was demonstrated for *Notch* mutant cells [2,3]. If the polar cells are wild type, however, normal egg chamber formation

Figure 1



The two rounds of Delta–Notch signaling during *Drosophila* oogenesis. A schematic drawing of an ovariole (at the top) shows the germarium and egg chambers of progressive developmental stage (S1 to S10) connected by interfollicular stalks. The oocyte of each egg chamber is at the posterior (right). Two boxes indicate the stages in which Delta–Notch signaling is likely to occur. **(a)** The first round of Delta–Notch signaling (blue arrows) takes place in the germarium and requires *fringe* expression (purple) in a subpopulation of follicle cells [2,3]. It comprises two signaling events [3] in which Delta (Dl) first signals from the germ line to specify polar cells. The polar cells in turn use Delta signaling to control stalk formation. **(b)** The second round of Delta signaling probably takes place during stages 5–7 [3]. Delta signaling from the germ cells is required for the differentiation of all epithelial follicle cells. The epithelial follicle cells become subdivided into two populations: the terminal (green) and the main body follicle cells (white) [10,11]. Only the terminal follicle cells are competent to respond to Gurken signaling by adopting posterior cell fates. By default, the terminal cells at the anterior pole of the egg chamber which do not receive the Gurken signal adopt anterior fates.

occurs even if the majority of all other follicle cells, including the stalks cells, are mutant. Thus, the polar cells organise cyst encapsulation and stalk formation.

Interestingly, the formation of the polar–stalk cell unit requires *Delta* to be present in both the germ line and the follicle cells [3]. Taking out *Delta* from the germ line results in the absence of polar cells and in a total failure of egg chamber formation. This indicates that egg chamber formation is guided by a germ line signal to the in-growing somatic cells. If the follicle cells are mutant for *Delta*, they are able to encapsulate the cysts, but stalk formation does not occur [3]. This suggests a relay model for the formation of the polar–stalk cell unit in which Delta signals from the germ line to induce the differentiation of polar cells, which in turn use Delta to control stalk formation (Figure 1).

Lineage analysis has shown that, from early on, the follicle cells fall into two populations [18]: one which stops dividing and appears to differentiate already in the germarium, while the other remains in an immature state until later stages of oogenesis. The early differentiating follicle cells are the precursors of the stalk cells and the polar cells. The first round of Delta signaling from the germ line controls the differentiation of this cell lineage (Figure 1). Cells of the other group have been termed epithelial follicle cells, as they form the monolayer follicular epithelium that surrounds each cyst [3]. They go through five rounds of mitosis after the egg chamber has left the germarium. Interestingly, the differentiation of this group of cells, and thus the development of all follicle cell types of the *Drosophila* ovary, also depends on Notch.

Mosaic egg chambers with *Notch* mutant follicle cells appear to develop normally as long as the two polar cells are wild type [2,3]. During mid-oogenesis, however, *Notch* mutant epithelial follicle cells do not stop dividing, lack normal morphogenetic movements and fail to turn on differentiation markers. All epithelial follicle cells, irrespective of their position in the egg chamber, appear to require *Notch* activity to switch from an immature to a differentiated state. As they remain immature, *Notch* mutant follicle cells are unable to respond to Gurken by expressing posterior differentiation markers. The posterior follicle cells are not specified and the anterior-posterior axis of the oocyte cannot be established.

Notch is thus not directly required for specification or patterning of the posterior cells, as had been suggested earlier [1,9], but rather acts as a general differentiation switch in all epithelial follicle cells. As in the case of polar cell specification, the Notch ligand Delta is provided from the germ cells. At the stage that the switch from immature to differentiated epithelial follicle cells takes place, Delta protein is strongly upregulated in all the germ cells and Notch protein concentrates apically in the abutting follicle cells [3].

Taken together, these new findings show that there are two types of germ line signal during *Drosophila* oogenesis: those mediated by Delta which exert temporal control, and those mediated by Gurken which exert spatial control of follicle cell development. Two rounds of Delta signaling control the specification of all major follicle cell types: first the polar and stalk cells, and subsequently the epithelial follicle cells. In both instances, Delta appears to be present uniformly in all germ cells, and spatial restriction either results from local *fringe* expression or is absent altogether. As Delta signaling induces the differentiation of epithelial follicle cells, it is a prerequisite for the Gurken signaling that patterns the follicular epithelium. In contrast to Delta, Gurken is asymmetrically localised in the germ line, first to the posterior pole and then to the dorsal side of the oocyte, and this defines the polarity of the body axes.

Delta–Notch signaling from germ line to soma represents a new way that the Notch pathway is employed in *Drosophila*. So far, the best-studied functions of *Notch* have been in patterning, either during lateral inhibition or boundary formation [19]. In these processes, Notch and its ligand(s) are present in the same epithelial layer, and signaling leads to the restriction of cell fates to a single cell or to a stripe of cells. During the second round of signaling in oogenesis, Delta present in the germ cells activates Notch simultaneously in about 1000 epithelial follicle cells to control their state of differentiation, not their patterning. Here, the *Enhancer of Split* complex, essential for lateral inhibition [20], is not a target of Notch activation [3], nor does *fringe* appear to be required for the late signal [2]. Thus, studying Notch signaling in oogenesis might lead to the isolation of new down stream targets and up stream activating factors for Notch. Moreover, a number of known genes which are required in the germ line and mutations of which cause *Notch* phenotypes might prove to be involved in Delta modification or presentation [21,22]. The best examples for *Notch* as a differentiation and proliferation signal have been described from the nematode *Caenorhabditis elegans* and vertebrates [19]. Now, *Drosophila* oogenesis might provide a new entry point to study this important aspect of *Notch* function.

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