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Organogenesis: Keeping in Touch with the Germ Cells

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DE-cadherin and its novel regulator, the transmembrane protein Fear of Intimacy, have been found to control the adhesive interactions between germline and somatic cells that lead to gonad formation in *Drosophila*.

Germ cells that will develop into eggs and sperm are the most precious possession of an organism, as they will give rise to the next generation, propagating the species. By contrast, all other cells of the body, collectively refered to as soma, will die. Multicellular organisms often form germ cells very early during development. Not surprisingly, the body also sets aside a group of somatic cells that associate with germ cells, leading to the formation of a specialized organ, the gonad, which supports germ cell survival and differentiation. New work by the laboratories of Mark Van Doren and Ruth Lehmann [1,2] has shed light on the adhesive mechanisms that contribute to formation of the gonads in the embryo of the fruit fly *Drosophila*.

Two-winged insects (diptera) such as *Drosophila* are an excellent example of the early divide between germline and soma. The first individual cells that form in the eggs of these animals are the primordial germ cells — somatic cells are formed only somewhat later in development. The primordial germ cells of dipteran embryos were termed 'pole cells' by the 19th century German zoologist August Weismann, because of their location at the posterior pole of the egg. Weismann proposed a strict segregation of germline and soma in his 'theory of the germplasm', but ironically he did not recognize pole cells as germline cells. Ilya Mechmikov, a contemporary of Weismann, discovered that pole cells are incorporated into the gonads and produce oocytes [3].

In *Drosophila*, as in many other animals, primordial germ cells originate at a location different from the region where the gonads develop, and so they have to migrate to be incorporated into the gonads [4]. In *Drosophila*, primordial germ cells are moved into an epithelial pocket formed by the posterior endoderm, and then they migrate through the epithelium — thereby entering the body cavity — and into the adjacent mesoderm on either side of the embryo, where they associate with a special group of about 30 mesodermal cells. These are the somatic gonad precursor cells which arise from three segmental units (parasegments 10, 11 and 12) and arrange as an elongated band of cells when the primordial germ cells make contact (Figure 1). The somatic gonad precursor cells ensheath the primordial germ cells and then condense into a round ball of cells, the embryonic gonad which is located in parasegment 10 [4]. Gonads can develop in the absence of primordial germ cells, suggesting that the forces that drive gonad formation originate in the somatic gonad precursor cells [1,5].

It has now been revealed that the condensation of gonadal cells into an ovoid ball, a morphogenetic process refered to as compaction or coalescence, involves two transmembrane proteins, DE-cadherin and Fear of Intimacy (Foi). DE-cadherin is a well-known molecule that mediates adhesion between neigboring cells through self-association (homophilic adhesion). DE-cadherin contributes to a variety of developmental processes that establish cell and tissue architecture or promote cell movement [6]. Embryos that do not express DE-cadherin show two distinct defects in their gonads: gonadal cells do not condense and remain an elongated band of loosely associated cells [1,2]; and the somatic gonad precursor cells fail to ensheath primordial germ cells [1]. During normal gonad development, individual primordial germ cells are wrapped in thin cellular processes which are extended by somatic gonad precursor cells. Ensheathment is initiated as soon as primordial germ cells contact somatic gonad precursor cells after their migration, and is maintained subsequently although the number of primordial germ cells increases through cell division [1]. The intimate contact between germline and somatic cells in the gonad facilitates the exchange of signals that are needed for the coordinated development of both cell types.

DE-cadherin is a member of the classic cadherin family of adhesion and signaling receptors which has over 20 orthologues in humans, including E-, N- and P-cadherin. Classic cadherins connect to the cytoskeleton via cytoplasmic adaptor molecules — the catenins — a linkage which is essential for effective cell adhesion [7]. Classic cadherins can promote cell sorting — the grouping of cells according to their adhesive properties. If two groups of motile cells that express different cadherins or different levels of the same cadherin are mixed, they sort themselves out. Cells with the highest level of adhesion form a tight round cluster that segregates away from, or is surrounded by, the other cells depending on how much adhesion is realized between the two groups of cells. As a result, cells adopt an arrangement that is energetically favorable and is characterized by the maximal amount of adhesion that can be established between cells [8].

Cell sorting among three different cell types may contribute to gonad formation. The primordial germ cells and the somatic gonad precursor cells both express DE-cadherin, whereas other mesodermal cells do not express this molecule [1]. While primordial germ cells continuously display DE-cadherin, somatic gonad precursor cells inititiate expression of DE-cadherin, dependent on the transcription factor Eyes

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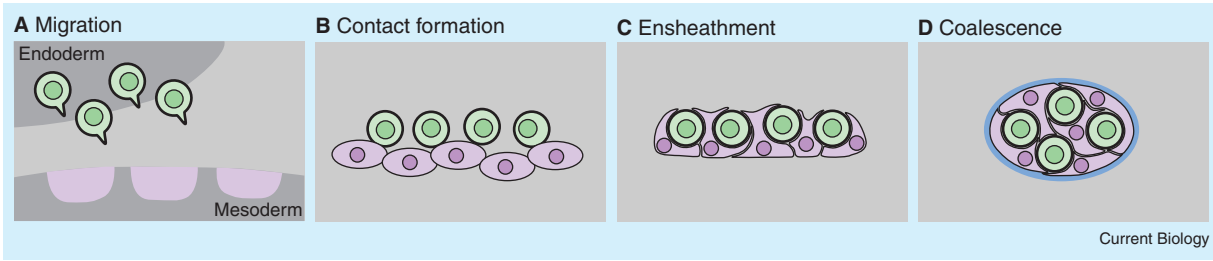

Figure 1. Steps in the formation of the *Drosophila* gonad.

(A) Primordial germ cells (green) migrate from the endoderm to the mesoderm, which contains three clusters of somatic gonad precursor cells (purple) in parasegments 10–12. (B) Primordial germ cells establish contact with somatic gonad precursor cells. (C) Somatic gonad precursor cells ensheath primordial germ cells. (D) The gonad coalesces into an ovoid ball in parasegment 10, surrounded by basement membrane (blue). Migration of primordial germ cells requires maternally provided DE-cadherin in the primordial germ cells. Ensheathment and coalescense require DE-cadherin and Foi expression in somatic gonad precursor cells. Coalescense can be driven solely by interactions between somatic gonad precursor cells, but ensheathment most likely also requires DE-cadherin expression in the germline.

Absent, shortly after they are specified. DE-cadherin expression allows somatic gonad precursor cells to segregate away from other mesodermal cells and to condense into a tight cluster. Ensheathment of primordial germ cells by somatic gonad precursor cells also requires DE-cadherin-mediated adhesion [1]. Wrapping of individual primordial germ cells by somatic gonad precursor cells would be promoted if adhesion between primordial germ cells and somatic gonad precursor cells is stronger than adhesion between either cell type alone. The importance of having correct amounts of DE-cadherin that enable ensheathment is illustrated by an experiment in which DE-cadherin levels are increased in the primordial germ cells, which prevents somatic gonad precursor cells from enveloping the germ cells [1]. Thus, differential adhesion mediated through DE-cadherin can explain aspects of gonad formation.

A question that remains is how the particularly strong adhesion between primordial germ cells and somatic gonad precursor cells is achieved if DE-cadherin is the only adhesion molecule mediating ensheathment. It is interesting that, although both cell types express DE-cadherin, it accumulates only at contact sites between primordial germ cells and somatic gonad precursor cells, and not between primordial germ cells, suggesting that the germline–soma interaction is stabilized by an additional, as yet unknown mechanism [1]. Moreover, the apparently low adhesion between primordial germ cells — in contrast to the adhesion between somatic gonad precursor cells, which is clearly substantial as these cells contact each other and drive compaction — should lead to an arrangement in which primordial germ cells are located in the periphery of the gonad and somatic gonad precursor cells forming an internal cluster. The observed reverse arrangement may result from adhesion of the somatic gonad precursor cells to the surrounding basement membrane, which maintains their peripheral position.

The *foi* gene was discovered in a genetic screen for mutations that disrupt gonad formation [9]; it encodes a protein with six, or possibly eight, transmembrane domains that localizes to the cell surface of germline and somatic cells of the gonad [2]. Removal of Foi from the germ cells has no obvious adverse effects, while the loss of Foi from somatic gonad precursor cells causes compaction and ensheathment to fail, and thus mimics the defects seen in embryos with decreased amounts of DE-cadherin. In *foi* mutants, the concentration of DE-cadherin is strongly reduced in somatic gonad precursor cells but not in most other tissues, suggesting that Foi is a somatic gonad precursor cell specific positive modulator of DE-cadherin, which readily explains its impact on gonad morphogenesis. Embryos that do not express Foi or DE-cadherin show also similar defects in the development of the tubular network of the respiratory system of flies, further supporting the notion that Foi interacts with DE-cadherin in selected cell types [2]. Foi belongs to a conserved family of proteins that is found in fungi, plants and animals including humans. The human orthologue of Foi, pLIV1, was implicated in breast cancer [10], as is E-cadherin, a well-known tumor suppressor [11], raising the possibility that the two factors may interact in humans. How Foi acts at the molecular level and maintains high amounts of DE-cadherin on the cell surface remains to be determined.

The contribution of DE-cadherin to germline development is an impressive example of how the activity of a single adhesion molecule can regulate multiple distinct aspects of organ development. Jenkins *et al.* [1] not only describe roles for DE-cadherin in gonad compaction and germ cell ensheathment, but also note that DE-cadherin is required for the migration of primordial germ cells from the endoderm to the mesoderm. This migration process has been studied intensively and is controlled by attractive and repulsive guidance cues [4]. How DE-cadherin contributes to primordial germ cell migration and interacts with the guidance machinery remains to be explored. In postembryonic gonad development, DE-cadherin contributes to the recruitment of some primordial germ cells as germline stem cells in the female ovary [12], and DE-cadherin is also implicated in the maintenance of follicle stem cells during oogenesis [13]. As oogenesis proceeds, DE-cadherin again mediates germline–soma interactions which first regulate a cell sorting process that positions the oocyte at the posterior pole of the ovarian follicle, and later

control the migration of groups of follicle cells over the surface of the much larger germline cells [6].

How cadherins, or adhesive mechanisms in general, participate in gonad development in other animals is still largely unexplored. Vertebrates express cadherins in primordial germ cells and surrounding somatic cells during migration and gonad formation. For example, zebrafish primordial germ cells and somatic gonadal cells express E-cadherin, and mammalian primordial germ cells express E-, N-, and P-cadherin [14,15]. Mammalian E-cadherin has been proposed to promote interactions between primordial germ cells that are required for the compaction of germ cells into a tight cluster contained within the developing gonad [15]. It will be interesting to see whether cadherins play equally diverse roles in vertebrate gonad development than in *Drosophila*.

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