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Signaling Reaches to New Dimensions in *Drosophila* Imaginal Discs

Minireview

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The field of developmental biology has cataloged a vast array of structures and processes in many different organisms, creating a terminology sufficient to portray the unique aspects that could be observed. Although the use of these different terms implies unrelatedness, the degree to which apparently different structures and processes are homologous has been uncertain. During the past several decades, the systematic application of developmental genetics has transformed developmental biology. Many of the genes that regulate development have been identified, and the processes they control can now be described in terms of signaling and regulatory pathways. One of the more remarkable findings to emerge from this new molecular understanding is that many pathways in distantly related organisms are homologous. Insect segmentation and the repetitive nature of the vertebrate body plan, once believed to be examples of convergent evolution, both are now understood to be regulated by related HOX gene action. Invertebrate compound eyes and vertebrate eyes, also thought to have been evolutionarily unrelated, are both created by developmental pathways that are regulated by homologous genes (reviewed in Gehring and Ikeo, 1999). Morphogens have also been identified that directly regulate growth and patterning in both vertebrates and invertebrates (e.g., Hedgehog (Hh), Wnt, BMP, and FGF). We can conclude that many apparent differences mask fundamental mechanistic similarities, and although we do not yet appreciate the full extent to which distantly related organisms share developmental mechanisms, it seems safe to predict that as we learn more about the processes that developmental biology describes, more of the commonality among organisms will be revealed.

We discuss two instances of commonality that have recently emerged and that are described in papers by Gibson and Schubiger (2000) and Cho et al. (2000) in this issue of *Cell*. Both groups studied signaling in *Drosophila* imaginal discs and made observations that lead them to propose that imaginal disc patterning involves signaling between cell layers. They also suggest that signaling *between* these cell layers occurs via long cytoplasmic extensions. As the possibility for vertical interactions in imaginal discs had never been considered previously, these suggestions are unexpected and open an exciting new dimension to this very active field.

Common Themes in Patterning

Studies of vertebrate embryos and fly imaginal discs have led to remarkably different conclusions about the nature of the patterning process. In vertebrates, interactions between distinct tissue layers have been implicated. There are several highly instructive and beautifully documented examples, most notably tooth and limb development. In these systems, communication between mesenchyme and ectoderm is bidirectional and essential for proper specification and development. In flies, all of the genetic interactions that pattern imaginal discs are thought to occur within the plane of epithelial sheets, and there has been little evidence to suggest that vertical interactions between tissue layers play any role. All current models are based on the assumption that patterning in imaginal discs is confined to two dimensions.

Imaginal discs form as groups of 10–50 cells that invaginate from the epithelium of the embryo and proliferate extensively during larval development. Throughout this period of growth, little overt differentiation occurs, although several different cell types can be distinguished in third instar discs (Figure 1A). Cells in the stalk region that connect the disc to the larval epithelium are clearly distinct. The remaining cells form into two juxtaposed monolayers. On one surface, a folded epithelium develops that is composed of columnar cells. These cells form the "disc proper," which in the wing disc produces the adult wing blade and thorax, in the eye disc produces the eye and most of the head capsule, and in the leg disc produces the leg. Cells on the other surface are larger, fewer in number, and squamous in shape; they contribute few adult structures. This region of the disc has been given the rather misleading moniker, "peripodial membrane." Cuboidal cells at the edge of the discs that lie between the disc proper and peripodial membrane constitute a fourth cell type (Usui and Simpson, 2000).

Studies of disc patterning have taught us much about the role of compartment borders as signaling centers and of how the Hh, Wingless (Wg), and Decapentaplegic (Dpp) morphogens control proliferation and cell fate in discs. Since the cells of the disc proper give rise to the principal adult structures, it is not surprising that attention has focused on them. In contrast, the peripodial cells are thought to primarily contribute a structural or morphogenetic role during metamorphosis. Their potential involvement in patterning has been largely ignored. This situation now changes with the work described in the above-mentioned papers. Cho et al. (2000) show that Hh, Wg, and Dpp are expressed in discrete and evolving patterns in the peripodial cells of developing eye discs and that expression of these genes in the peripodial cells is required for controlling expression of the Notch ligands Delta and Serrate in the disc proper. Gibson and Schubiger (2000) show that the cells in the disc proper do not develop normally if peripodial cells are killed or if peripodial cells ectopically express *fringe* or a dominant-negative form of Serrate. The observations of both groups suggest that peripodial cells are required for the development of the disc proper, implying that peripodial cells signal to the disc columnar cells.

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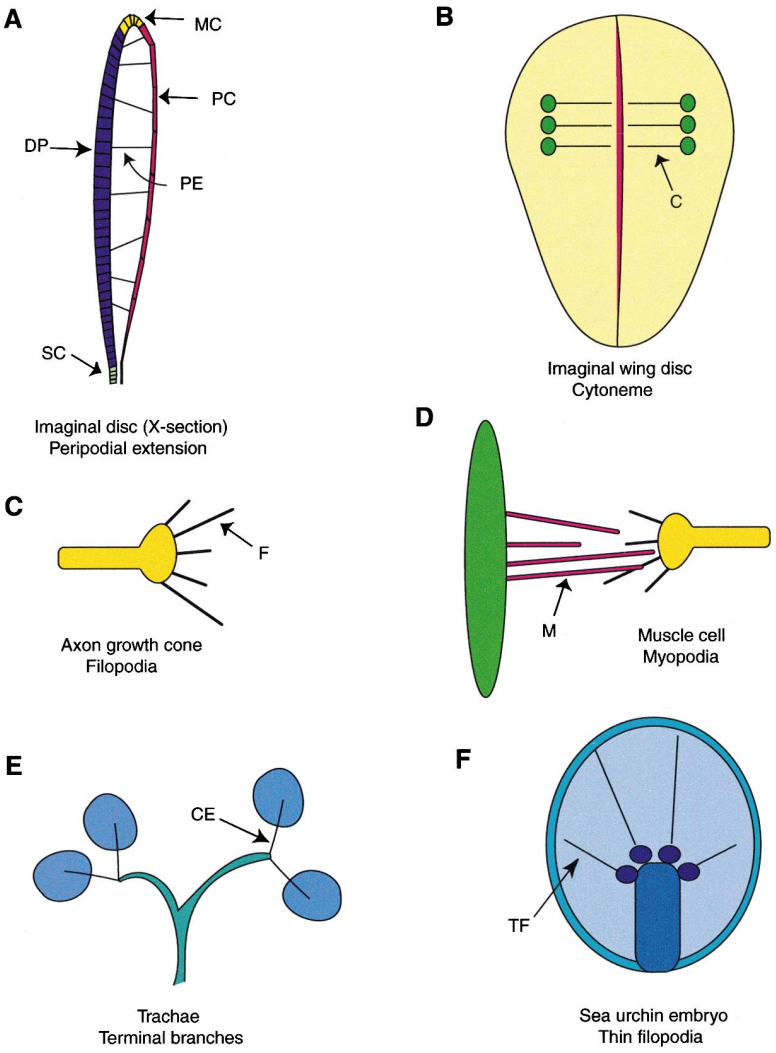

Figure 1. Common Structures in Cells that Respond to Long-Range Patterning Cues

(A) *Drosophila* imaginal discs flatten to form two monolayers—one called the disc proper (DP) and the other populated by peripodial cells (PC). Stalk cells (SC) connect the disc to the embryo or larval epidermis, and margin cells (MC) lie between the DP and PC. Peripodial cells make long microtuble-based cytoplasmic extensions (PE) to the columnar cells in the disc proper. These structures may transmit signals between the cell layers.

(B) *Drosophila* imaginal discs cells make polarized actin-based extensions (C, cytonemes) that connect them to the anterior/posterior organizing center. Cytonemes may transmit signals between the organizing center and the outlying cells.

(C) Actin-based filopodia (F) extend from the tip of a neuronal growth cone. Filopodia are thought to guide the growing axon to the proper target by sensing extracellular cues.

(D) Embryonic muscle cells extend actin-filled extensions (M, myopodia) that contact the growth cone of an approaching motor neuron and cluster at the site of synapse formation. They may have a role in guiding the axon to the site of enervation.

(E) *Drosophila* responds to cells that are oxygen-deprived (blue forms) by extending terminal branches of tracheal cells. Terminal branches arise as long, thin cytoplasmic extensions (CE), presumably in response to a chemoattractant produced by the target cell.

(F) Primary mesenchyme cells (PMC) of the sea urchin embryo extend actin-based extensions (TF, thin filopodia) to the overlying ectoderm. These structures may transmit signals needed by the PMC to produce a normal skeleton.

This bold and novel suggestion is an exciting new development that will undoubtedly inspire more investigations into the roles and properties of the peripodial cells. We can hope that these studies will broaden our understanding of tissue patterning—that they might reveal, for instance, if interactions between disc and peripodial cells are bidirectional, how cell types are assigned in patterned arrays, and how discs set up secondary axes during larval development, all questions that have eluded solution to date.

These are also obvious parallels to vertebrate patterning. In vertebrate embryos, the visceral endoderm is an extraembryonic tissue that overlays the embryo and had been considered to be irrelevant to the processes that organize and pattern the embryo. Recent observations now suggest that interactions between the visceral endoderm and the embryo are essential for patterning, especially for anterior structures such as the forebrain (reviewed in Beddington and Robertson, 1998). So it may be that interactions between the disc and peripodial cell layers in insects are intrinsically similar to interactions between cell layers in vertebrates, broadening the significance of studies into their basis and role.

Common Signaling Strategies

Cell communication can be mediated by direct contact or indirectly by secreted substances. With the exception of nerve cells, long distance communication has been thought to be accomplished solely by moving secreted molecules in extracellular spaces. In part, this view has been based on the belief that neurons are uniquely endowed with cell processes that reach out long distances from the cell body. The cell–cell contacts of all other cells have been thought to be confined to nearest neighbors. We suggest that this view is incorrect. Work in a number of laboratories as well as the present work leads us to suggest that most or all cells have the capacity to generate long processes which are used to explore the extracellular environment. Neurons may have acquired the ability to differentiate such processes for their unique purposes, but the ability to make direct contacts over long distances is not solely a neuronal trait.

We were alerted to this possibility when our studies of wing discs detected long actin-based extensions (cytonemes) that project toward the A/P signaling center from outlying cells (Figure 1B; Ramírez-Weber and Kornberg, 1999). The polarity and orientation of cytonemes relative to the principal signaling center in the disc is

highly suggestive of a role in communicating signals over long distances. Perhaps target cells do not wait passively for instructions, but reach out to make contact with the sources of their signals. This model of cell–cell communication has the attractive attribute that it offers opportunities for specificity and regulation that would be lost if the signaling molecules are dispersed to find their targets by random means. But it remains to be established what their function is and how widespread they are.

Although cytonemes had not been observed previously in imaginal discs, cell extensions that are thought to have a role in signaling during development have been observed in other contexts (see Figures 1C–1F). Since it is possible that these various structures have similar functions, they might represent different adaptations that serve a common purpose. The following is a brief description of the various systems in which cell extensions have been observed.

In insects, cell extensions have been found in several different tissues. In *Drosophila* leg, wing, and eye imaginal discs, cytonemes grow along the apical surface of the disc cells (Ramírez-Weber and Kornberg, 1999). In *Drosophila* egg chambers, long cell extensions connect nurse cells with migrating border cells (Goode, 2000). Although larger in diameter than disc cytonemes, these processes are also actin-filled and are also polarized toward an organizing influence. Goode suggests that the germ cell extensions may help guide the migrating border cells by funneling signals that induce cell movement. Other examples of filopodial extensions of insect cells have been described by Locke (1987). He has shown that many insect cells, including epidermis, fat body, oenocytes, and pericardial cells can be induced to form multiple thin (0.1 μm diameter) filopodia that extend for 10–30 μm if their contacts with neighboring cells are severed. These filopodia retract and disappear after proper attachments have been restored. Their function is not known.

Filopodia are also present in the growth cones at the termini of axons (Figure 1C). Growth cones have an expansive central domain that is richly endowed with microtubules. At the leading edge of growth cones, a highly dynamic peripheral region extends and retracts actin-filled filopodia. These filopodia are thought to play an active role in synaptic targeting, in part by serving as antennae that scout in advance of axonal growth. In embryonic grasshopper limbs, microtubules have been observed to selectively invade branches derived from filopodia that had contacted appropriate targets (Sabry et al., 1991). This observation suggests that growth cone filopodia may serve as foundations for axonal growth and that actin- and tubulin-filled extensions might have a common origin.

Muscle cells have also been found to make dynamic filopodia (Figure 1D). Recently obtained evidence indicates that muscle cells in *Drosophila* embryos extrude numerous actin-filled filopodia (myopodia) that intermingle with growth cone filopodia and cluster at the site of motoneuron enervation (Ritzenthaler et al., 2000). Myopodia appear to contribute to growth cone guidance, and their active clustering in response to the approach of a growth cone suggests as well that communication between the neuron and its target is bidirectional.

Long cytoplasmic extensions of tracheal cells have been observed in a number of different insects (Figure 1E; Wigglesworth, 1977; Jarecki et al., 1999). The main trunks of the insect tracheal system are stererospecifically determined by a genetically controlled pathway, but terminal branches form elaborate networks that reach out in an apparently stochastic manner to oxygen-deprived cells. These terminal branches grow toward target tissue, arising from long, thin (0.1–1 μm diameter) cytoplasmic extensions (Jarecki et al., 1999). The structural or functional relationship between these terminal branches and other cell extensions is not known, but there are obvious parallels with the cytonemes and with the maturation of growth cone filopodia.

Sea urchin embryos are remarkably transparent, an important attribute that contributed to the finding that long and very thin "pseudopodia" extend from primary mesenchyme cells (Figure 1F; reviewed in Gustafson and Wolpert, 1967). During sea urchin development at a stage when the embryo is organized as a sphere of cells, primary mesenchyme cells involved in spicule formation migrate inside the sphere. These primary mesenchyme cells make filopodia that are highly dynamic and appear to contact and explore the overlying ectoderm. These thin filopodia have dimensions similar to cytonemes; they are also actin-based and extend and retract rapidly. Their function as conduits of information is suggested by the instructive role that the ectoderm plays in patterning the skeleton and the fact that under conditions in which the thin filopodia are disturbed, skeleton development is abnormal (reviewed in McClay, 1999).

The work of Cho et al. (2000) and Gibson and Schubiger (2000) now expands the inventory of cell extensions in fly imaginal discs. Both groups observed processes extending out from peripodial cells. Cho et al. (2000) found evidence for processes that are oriented from peripodial cells to the disc proper. Gibson and Schubiger show that peripodial cells have tubulin-filled processes that project toward disc columnar cells, terminating at or near the surface of the columnar epithelium. Since both groups have also shown that development of the disc cells is influenced by the expression of several key signaling proteins in the peripodial cells, the presence of these extensions leads both groups to propose a role for cell extensions in translumenal signaling.

Summary

Finding that peripodial cells in wing and eye imaginal discs are essential for the growth and patterning of the separate layer of disc cells now opens the study of interacting cell layers to the powerful developmental genetic techniques with which the *Drosophila* system is blessed. We can anticipate that future work will identify how such interactions contribute to patterning and how the mechanisms and processes that are involved are conserved in vertebrates. We can also look forward to contributions that this work will make to understanding the role of interconnecting cell extensions in such signaling processes. In this minireview, we have noted numerous types of signaling cells in which cellular extensions have been observed. At present, neither the functional nor structural relationship of these related structures is known. It is certainly tempting to suggest that these structures are conduits for signals or that they

function as sensors. There is, as yet, no direct experimental evidence for such roles.

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