

Drosophila peripodial cells, more than meets the eye?

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Summary

Drosophila imaginal discs (appendage primordia) have proved invaluable for deciphering cellular and molecular mechanisms of animal development. By combining the accessibility of the discs with the genetic tractability of the fruit fly, researchers have discovered key mechanisms of growth control, pattern formation and long-range signaling. One of the principal experimental attractions of discs is their anatomical simplicity — they have long been considered to be cellular monolayers. During larval stages, however, the growing discs are 2-sided sacs composed of a columnar epithelium on one side and a squamous 'peripodial' epithelium on the other. Recent studies suggest important roles for peripodial epithelia in processes previously assumed to be confined to columnar cell monolayers. *BioEssays* 23:691–697, 2001.

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Introduction

In most primitive insect groups, the appendage primordia arise as evaginations or buds on the outside of the developing embryo. It is only in the more-derived holometabolous species, such as *Drosophila*, that some or all of the primordia of adult structures are sequestered in epidermal invaginations called imaginal discs. In *Drosophila* larvae, imaginal discs are flattened epithelial sacs with two opposing surfaces, a columnar epithelium and a squamous peripodial epithelium (or peripodial membrane).⁽¹⁾ Since the first disc fate maps were produced⁽²⁾ and through to the molecular genetic dissections of the present day, investigations of disc development have focused on the more numerous cells of the columnar epithelium. Reasons for this emphasis are several. The thickened and opaque disc columnar epithelia are much more easily visible than the squamous peripodial sheet. More importantly, during metamorphosis, disc columnar cells directly produce the vast majority of the adult cuticle while peripodial cells make only a trifling contribution.

As a result of these and other factors, *Drosophila* imaginal discs are often described as monolayered columnar epithelia. Although the peripodial and columnar epithelia literally constitute a contiguous cell sheet, the term monolayer does

not accurately reflect the sac-like anatomy of the imaginal discs. Here we consider the often-ignored (and poorly named) peripodial 'membranes'. Might their function extend beyond that of simple sheaths to contain developing discs? It has been known for some time that peripodial cells function in metamorphosis,^(3,4) but more recent studies indicate that they could also regulate earlier phases of disc development. In reviewing the existing literature concerning the function of peripodial cells, we will advance the hypothesis that, despite a minimal direct contribution to the adult fly, peripodial cells figure prominently in larval and metamorphic development. For the purposes of this review, the term peripodial epithelium will be used in place of the historical but misleading designation peripodial membrane. We recommend that this change in nomenclature apply generally to future work with imaginal discs and note that 'peripodial epithelium' is already the standard designation employed by the 'Flybase' online *Drosophila* database.⁽⁵⁾

Our discussion begins with adult morphogenesis at the onset of metamorphosis, where peripodial epithelia have a reasonably well-defined function. Imaginal discs develop internally in the larva and, during metamorphosis, a radical morphogenic transition externalizes the discs and assembles the adult de novo. During this stage, discs evert from within their peripodial sacs (disc eversion) and then fuse with adjacent disc derivatives (disc fusion) to form a continuous adult epidermis.⁽⁶⁾ While some insightful older studies have considered the mechanisms of disc eversion,^(3,4,6,7) more recent work has brought to light some of the cellular and molecular events underlying disc fusion.^(8–10) Peripodial cells play crucial roles in both processes.

Disc eversion

Metamorphic events in *Drosophila* imaginal discs have been reviewed in detail.⁽⁶⁾ In the mid-third instar, the steroid molting hormone 20E (hereafter referred to as ecdysone) induces the discs to undergo a series of complex metamorphic changes, generally termed evagination. Evagination involves two discrete processes: elongation, during which morphogenetic events in disc columnar epithelia cause lengthening and shaping of the appendages themselves, and eversion (Fig. 1A–D), wherein contraction of peripodial epithelia is thought to drive movement of the appendage to the outside of the larval epidermis.⁽⁶⁾ As eversion occurs almost simultaneously in all discs, it could be assumed to be globally

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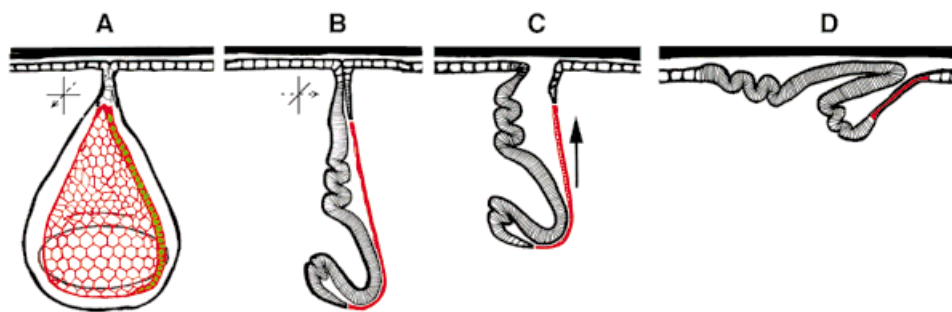


Figure 1. Wing disc eversion. **A:** The traditional two-dimensional view of a wing imaginal disc usually does not represent the squamous peripodial membrane cells, which are shown here in red. The green cells are peripodial ‘medial edge’ cells. **B:** In cross section, the peripodial membrane is apparent (red), as well as the luminal cavity between the two cell layers. **C, D:** During disc eversion, peripodial cells contract, forcing the columnar epithelium to the outside of the body. Modified from Fristrom and Fristrom (1993).⁽⁵⁾

coordinated by the late third instar peak of ecdysone. Indeed, *in vitro* work by Milner et al.⁽³⁾ showed that treatment of cultured eye discs with ecdysone is sufficient to induce eversion, and that contraction of an intact peripodial epithelium is necessary for disc eversion. Additional studies indicate that disc eversion *in vitro* requires some minimal titer of ecdysone.⁽⁷⁾ The contraction–eversion functionality of peripodial epithelia appears to be conserved in the appendage primordia of other holometabolous groups, such as the wing discs of the Lepidopteran *Manduca sexta*. Nardi et al.⁽¹¹⁾ report that evagination of the *Manduca* wing disc is coincident with a columnarization of cuboidal peripodial cells, which radically reduces the surface area of the peripodial cell sheet, possibly driving eversion. The authors propose that such cell shape changes could be a general mechanism of epithelial morphogenesis.

Together the above studies define a role for peripodial epithelia in disc eversion, although there are as yet no data to confirm that this mechanism exists *in vivo*. The combined results of Milner et al.^(3,4) and Guillermet and Mandaron⁽⁷⁾ imply that high titres of ecdysone could be responsible for inducing peripodial cell shape changes, leading to externalization of the appendage primordia during early pupal development. This idea has not been directly explored on a molecular level, but is supported by the expression of various genes of the ecdysone regulatory cascade in peripodial epithelia (for example, EcRB-1,⁽¹²⁾ E74A,⁽¹³⁾ DHR78,⁽¹⁴⁾ broad complex-Z1 isoform,⁽¹⁵⁾ and Imp-L2⁽¹⁶⁾). We suggest that cytoskeletal reorganization drives contraction of the peripodial cells, and therefore propose a functional link between ecdysone signaling, the control of peripodial cell architecture and adult morphogenesis. Consistent with this idea, animals mutant for the EcR-B1 isoform of the ecdysone receptor fail to evert their discs during metamorphosis.⁽¹⁷⁾

Disc fusion

Following evagination, neighboring discs fuse (disc fusion) to form the continuous adult epidermis. Again, this topic has been reviewed by Fristrom and Fristrom⁽⁶⁾ and has been considered in the more recent work of Usui and Simpson.⁽¹⁰⁾ Here we will focus on peripodial cell function in fusion of the wing imaginal discs — a process known as thorax closure (Fig. 2) that has been studied extensively on a molecular-genetic level. Mutants in *hemipterous* (*hep*), which encodes the *Drosophila* JNK-Kinase,⁽¹⁸⁾ display abnormal thorax closure resulting in a distinctive ‘split thorax’ phenotype.^(8,9) A similar phenotype is observed in mutations in the transcription factor *dFos*.^(9,19) Together, these mutant phenotypes provide strong evidence for the involvement of the JNK signaling pathway in thorax closure along the dorsal midline. These genes also regulate the process of embryonic dorsal closure (where the left and right sides of the embryonic epidermis fuse along the

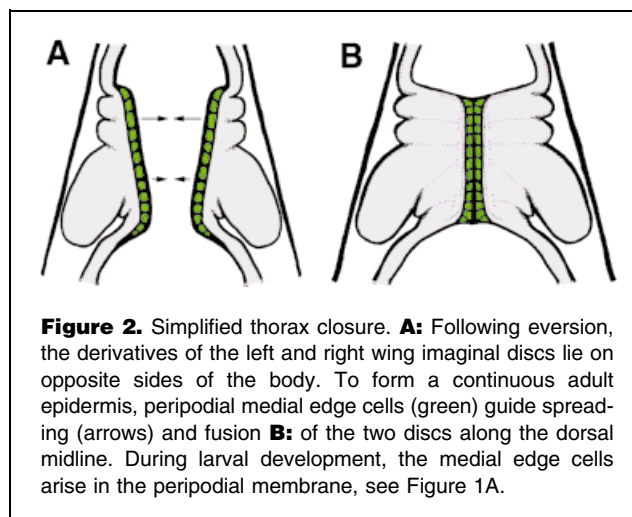


Figure 2. Simplified thorax closure. **A:** Following eversion, the derivatives of the left and right wing imaginal discs lie on opposite sides of the body. To form a continuous adult epidermis, peripodial medial edge cells (green) guide spreading (arrows) and fusion **B:** of the two discs along the dorsal midline. During larval development, the medial edge cells arise in the peripodial membrane, see Figure 1A.

presumptive larval dorsal midline, Ref. 20). Based on these similarities, it has been suggested that a conserved mechanism regulates the spreading and fusion of epithelial sheets at two distinct stages in *Drosophila* development.

The similarities between dorsal closure in the embryo and thorax closure in the pupa extend to the cellular level. A specific subpopulation of peripodial ‘medial edge’ cells are crucial players in thorax closure.^(8,9) In embryonic dorsal closure, *hep* induces expression of *puckered* (*puc*; a negative regulator of the JNK pathway) in a narrow band of cells at the boundary between squamous amnioserosal and columnar epidermal cells. In late-larval wing discs, *hep*-dependent *puc* expression is detected in a stripe of peripodial medial edge cells that define the boundary between squamous peripodial cells and columnar epithelial cells of the disc proper.^(8,9) Hence in both dorsal closure and thorax closure, *puc* is expressed in boundary cells at a squamous/columnar interface. The significance of this boundary expression remains unknown, but should provide fertile ground for further inquiry.

Peripodial cell morphology

In the embryonic development of vertebrate limbs and eyes, interepithelial signaling (between tissue layers) is a central feature of both pattern formation and growth control. This contrasts sharply with what is known about *Drosophila* imaginal discs, where growth control and pattern formation are thought to be governed by planar signaling within cellular monolayers. We will now consider evidence that peripodial membrane cells signal ‘vertically’ to underlying disc columnar cells during larval growth and pattern formation. Morphological analysis of peripodial cells is entirely consistent with this proposition (Fig. 3). Three-dimensional confocal imaging of live peripodial cells shows that they produce highly specialized ‘translumenal extensions’ — filopodia that traverse the disc lumen and project toward the apical surface of the columnar epithelium.^(21,22) The subcellular organization of peripodial cells also hints at signaling between the two cell layers of the imaginal disc. Translumenal extensions contain a funnel-shaped internal membrane (the perinuclear lobe; Fig. 3B,C) which originates on the cell’s nuclear membrane, and could therefore represent a mechanism for targeting RNA or proteins into translumenal extensions. The extensions also contain microtubules and densely packed mitochondria, evidencing the structural and energetic capacity for directed transport.⁽²¹⁾ We also note that the apical surfaces of both epithelia, where the components of many signaling pathways are localized, are in direct face-to-face contact throughout larval development. It is therefore at least structurally plausible that peripodial and columnar cells communicate during disc development. Finally, two lines of correlative evidence indicate direct coordination between the two cell layers of the imaginal disc: (1) peripodial membranes are always properly fitted to their opposing columnar epithelium, implying coordinated growth/size con-

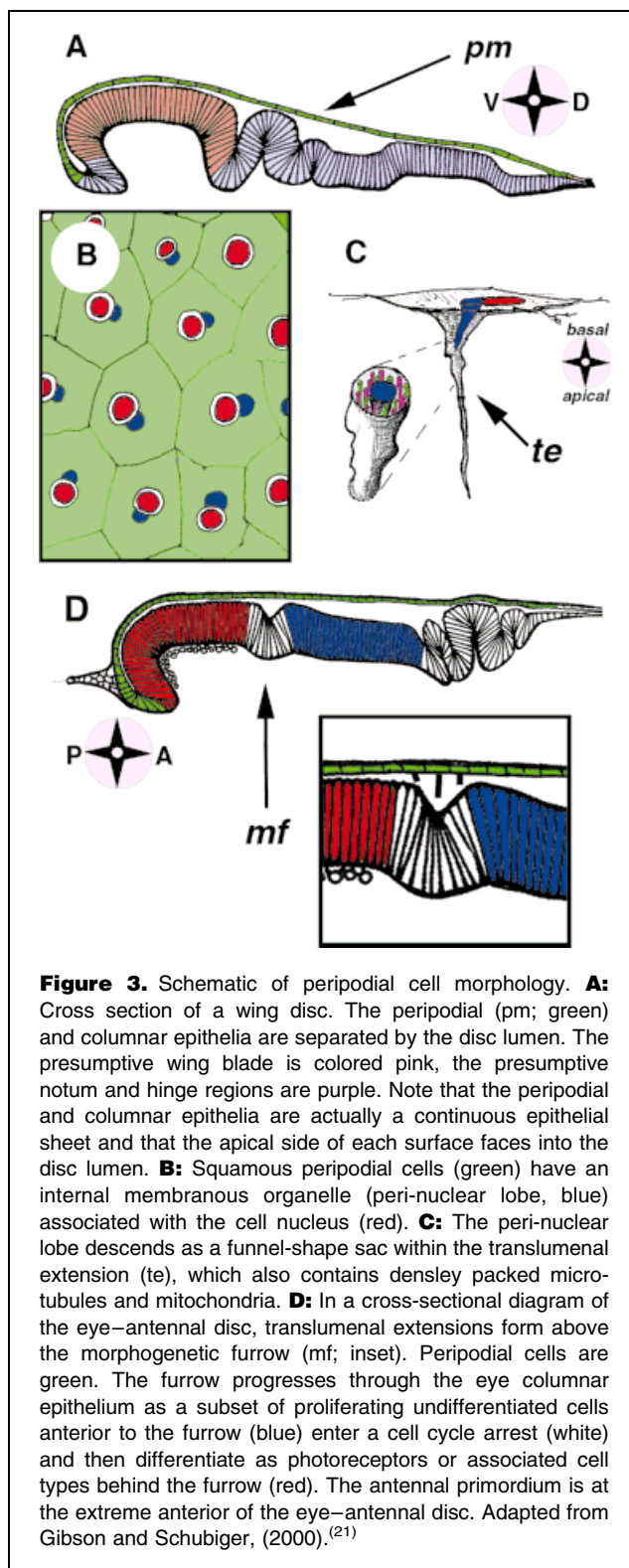


Figure 3. Schematic of peripodial cell morphology. **A:** Cross section of a wing disc. The peripodial (pm; green) and columnar epithelia are separated by the disc lumen. The presumptive wing blade is colored pink, the presumptive notum and hinge regions are purple. Note that the peripodial and columnar epithelia are actually a continuous epithelial sheet and that the apical side of each surface faces into the disc lumen. **B:** Squamous peripodial cells (green) have an internal membranous organelle (peri-nuclear lobe, blue) associated with the cell nucleus (red). **C:** The peri-nuclear lobe descends as a funnel-shaped sac within the translumenal extension (te), which also contains densely packed microtubules and mitochondria. **D:** In a cross-sectional diagram of the eye–antennal disc, translumenal extensions form above the morphogenetic furrow (mf; inset). Peripodial cells are green. The furrow progresses through the eye columnar epithelium as a subset of proliferating undifferentiated cells anterior to the furrow (blue) enter a cell cycle arrest (white) and then differentiate as photoreceptors or associated cell types behind the furrow (red). The antennal primordium is at the extreme anterior of the eye–antennal disc. Adapted from Gibson and Schubiger, (2000).⁽²¹⁾

trol, and (2) cell divisions in the disc columnar epithelia appear spatiotemporally coordinated with mitotic figures in the peripodial membrane.⁽²³⁾

Peripodial cells and disc regeneration

The initial evidence that peripodial and columnar cells actually communicate came from analysis of fragmentation-induced regeneration in the leg imaginal disc. Upon wounding and culture, imaginal discs grow and can regenerate missing structures or duplicate existing ones.^(2,24) For example, if the prothoracic leg disc is cut into an anterior-only '1/4' piece and a cognate '3/4' piece, the 1/4 fragment will regenerate a complete leg (both anterior and posterior) and the 3/4 piece will duplicate itself with mirror-symmetry. In electron microscopic studies of regenerating wing disc fragments, Reinhardt et al.⁽²⁵⁾ reported a transient heterotypic contact between peripodial and columnar cells at the site of wound healing. Based on recent experiments from our laboratory, this contact also appears to occur in 1/4 fragments of the first leg disc where some peripodial cells (and no columnar cells) express the secreted signal Hedgehog (HH). Using a temperature-sensitive allele to study the 1/4 fragments, we found that HH was required for normal regeneration of posterior structures in the disc columnar epithelium.⁽²⁶⁾ Because *hh* is only expressed in peripodial cells in these fragments, we were forced to conclude that HH signal produced by peripodial cells stimulates the regeneration of posterior cellular identity in anterior-only disc fragments. The only alternative explanation is that peripodial cells move into the columnar epithelium and 'seed' a novel posterior compartment, but clonal analyses exclude this possibility.^(26,27) Hence work on disc regeneration establishes a special case of peripodial-to-columnar communication in disc fragments.

Peripodial cells and 'normal' development

Genetic analysis is the greatest strength of the *Drosophila* model system, but over the decades-long investigation into disc development only a few genes have been noted to be expressed in peripodial epithelia (Table 1). Some of these observations, mostly made in passing, could lead to molecular-genetic evidence of communication between the peripodial and columnar epithelia. Indeed, recent results from Cho et al.⁽²²⁾ show that peripodial cells are subdivided into dorsal and ventral territories as early as the first larval instar and that some well-known molecular signals (*wingless* (*wg*), *hedgehog* (*hh*), and *decapentaplegic* (*dpp*)) are expressed in both peripodial and columnar cells during proliferative growth of the eye disc (Fig. 4). The dorsoventral subdivision indicates that peripodial epithelia are organized, and the expression of signaling molecules suggests a potential mechanism for the transmission of positional information between peripodial and columnar cells. Using a temperature-sensitive loss of function allele, Cho et al.⁽²²⁾ found that inactivation of *hh* during early development (when it is primarily expressed in the peripodial

membrane) results in aberrant expression of the Notch ligands Delta and Serrate in the underlying eye columnar epithelium. Further exploring *hh* function, the authors show that *hh* loss-of-function clones in the peripodial epithelium can elicit developmental defects in disc columnar cells. It remains to be seen whether the observed developmental defects reflect failures in peripodial–peripodial signaling, peripodial–columnar signaling, or both, but they clearly reveal peripodial–columnar interaction on some level. The results underscore the relevance of spatially and temporally dynamic gene expression patterns and the significance of the peripodial–columnar dichotomy. For any given gene, appendage phenotypes may reflect defects in peripodial cells, columnar cells, or both.

Peripodial cells have been implicated in both growth and patterning during eye development. The eye disc is patterned by progression of the morphogenetic furrow⁽³³⁾, a wave of proliferation and patterning that sweeps across the disc during the late third instar and into early pupal development. As the furrow passes, undifferentiated columnar cells assume specific identities (photoreceptors, cone cells etc.) and become recruited into nascent ommatidial clusters.⁽³⁴⁾ Recent evidence from our lab, both functional and descriptive, suggests that peripodial cells are important for furrow progression. We became interested in this topic because peripodial transluminal extensions were observed directly above the morphogenetic furrow⁽²¹⁾ (see Fig. 3D). This implies

Table 1. Genes expressed in peripodial cells

Gene	Type	Reference	Disc
Wingless	Signal	22	Eye
Decapentaplegic	Signal	22	Eye
Hedgehog	Signal	22	Eye
Eyeless	Transcription factor	21	Eye
Mirror	Transcription factor	(a)	Eye
Ultrabithorax	Transcription factor	28	Wing
Notch	Receptor	29	Eye
Serrate	Signal	21	Eye
Discs large	Cytostructural	22, (a)	Eye, wing
Puckered	Protein phosphatase	8, 9	Wing
DFos	Transcription factor	9	Wing
Homothorax	Transcription factor	30	Wing
Broad complex	Transcription factor	15	Eye
Imp-12	Cell adhesion	16	Wing
<i>Eip74EF (E74A)</i>	Transcription factor	13	Wing
<i>Hr78</i>	Hormone receptor	14	All?
<i>EcRB-1</i>	Hormone receptor	12	All?
Delta	Signal	29	Eye
Labial	Transcription factor	31	Eye
Deformed	Transcription factor	31	Eye
Sine oculis	Transcription factor	32	Wing
Scabrous	Structural protein	(b)	Eye

(a) M. Gibson, unpublished obs.

(b) Nicholas Baker, pers. comm.

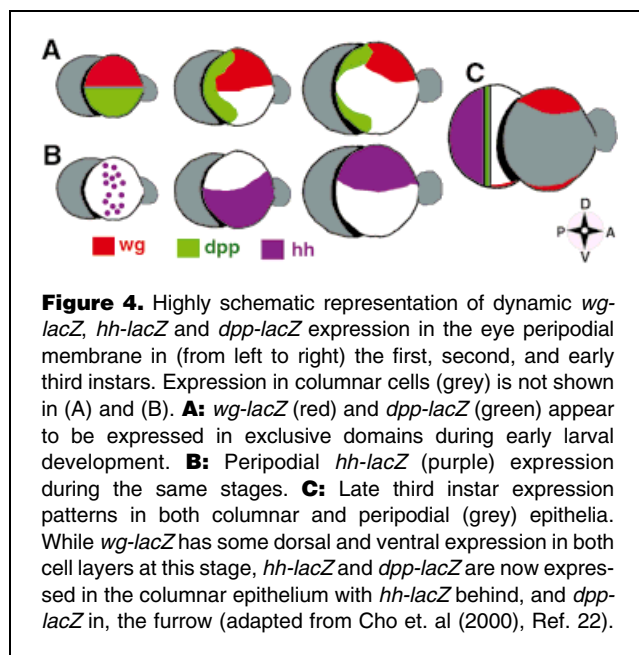


Figure 4. Highly schematic representation of dynamic *wg-lacZ*, *hh-lacZ* and *dpp-lacZ* expression in the eye peripodial membrane in (from left to right) the first, second, and early third instars. Expression in columnar cells (grey) is not shown in (A) and (B). **A:** *wg-lacZ* (red) and *dpp-lacZ* (green) appear to be expressed in exclusive domains during early larval development. **B:** Peripodial *hh-lacZ* (purple) expression during the same stages. **C:** Late third instar expression patterns in both columnar and peripodial (grey) epithelia. While *wg-lacZ* has some dorsal and ventral expression in both cell layers at this stage, *hh-lacZ* and *dpp-lacZ* are now expressed in the columnar epithelium with *hh-lacZ* behind, and *dpp-lacZ* in, the furrow (adapted from Cho et. al (2000), Ref. 22).

coordination between peripodial and columnar cells in the furrow, and suggests that peripodial transluminal extensions could have a furrow-specific function. Accordingly, we found that surgical ablation of the eye peripodial membrane disrupted furrow progression in cultured third instar discs. Similarly, when the eye peripodial membrane was genetically ablated with targeted expression of a toxic transgene, animals with small unverted eyes with irregular pattern were obtained. These ablation experiments strongly indicate that an intact peripodial epithelium is required for normal development of the *Drosophila* retina.⁽²¹⁾

Further experiments addressed the requirement for peripodial cell function without destroying the peripodial epithelium. We used the Gal4/UAS system⁽³⁵⁾ and an eye peripodial-specific Gal4 driver to express a dominant-negative form of Glued,⁽³⁶⁾ a component of the dynactin motor complex.⁽³⁷⁾ The intent of this experiment was to disable microtubule-based transport along the microtubule cores of eye disc transluminal extensions. As a result, we observed a marked reduction and/or loss of the mitotic waves normally observed both anterior and posterior to the morphogenetic furrow in the eye columnar epithelium. The results can be interpreted in one of two ways. First, peripodial-specific expression of dominant-negative Glued may have generally disrupted peripodial cell architecture, producing a non-specific (secondary) phenotype in furrow progression. This could be the case if peripodial Glued was normally required to regulate events prior to furrow movement, such as proliferative growth. Alternatively, the results could indicate that microtubule-based transport is required to move a peripodial signal through the transluminal extensions to the surface of the retinal primordium. We note

that, in either scenario, the results show that peripodial cell function is required for furrow progression in the disc columnar epithelium.

Understanding peripodial cell function in eye development will require a better understanding of the molecular agents involved, particularly the specific signals employed in transluminal communication. Some of the molecules involved are likely to be among those already known to have a role in eye development (see Table 1). For example, observations in the literature suggest that the Notch pathway is involved in transluminal communication. The compound eye of *Drosophila* is completely derived from the columnar epithelium; eye disc peripodial cells give rise to some structures of the adult head.⁽³⁾ Paradoxically, the Notch ligand Serrate is known to be required for eye development,⁽³⁸⁾ but *Serrate*⁻ (loss of function) cell clones in the eye columnar epithelium appear to be phenotypically normal.⁽³⁹⁾ The requirement for Serrate in eye development could lie in the peripodial epithelium. Consistent with this, Serrate is expressed at relatively high levels in peripodial cells and peripodial-specific misexpression of a secreted dominant-negative form of Serrate (using the Gal4/UAS system, Ref. 35) leads to a rough and reduced eye phenotype.⁽²¹⁾ Other evidence for involvement of the Notch pathway in transluminal signaling comes from analysis of the Notch-modifying glycosyltransferase Fringe,^(40–43) which is expressed in a subset of peripodial cells during early larval development.⁽²¹⁾ *fringe*⁻ (loss of function) clones in the columnar epithelium grow normally, but occasionally animals that contain *fringe*⁻ clones have reduced eyes.⁽⁴³⁾ Papayanopoulos et al. (1999) suggest that this size reduction reflects a requirement for Fringe somewhere outside the eye columnar epithelium. That Fringe acts on Notch signaling in the peripodial epithelium to control disc growth is a definite possibility.

Looking down on discs: peripodial speculation

At present, there are clearly more questions than answers with respect to peripodial cell function in disc development. The findings discussed above should lead us to consider the possibility of peripodial membrane function in processes previously assumed to occur within columnar epithelia (Fig. 5). Thinking along these lines must be tempered by the fact that insects produced limbs and eyes long before the first peripodial membrane enclosed an appendage primordium. We suggest that, in *Drosophila*, developing disc columnar epithelia may use their peripodial ‘neighbors’ to economize or refine ancestral developmental programs. This could be the case, for example, in the positioning of R8 ommatidial precursors in the eye columnar epithelium. Establishment of these structures and their proper spacing is generally thought to occur via lateral inhibition between neighboring cells in neurogenic regions of the columnar epithelium.^(44,45) However, the mechanism of R8 spacing is not perfectly understood. It is interesting to note that the spacing of peripodial

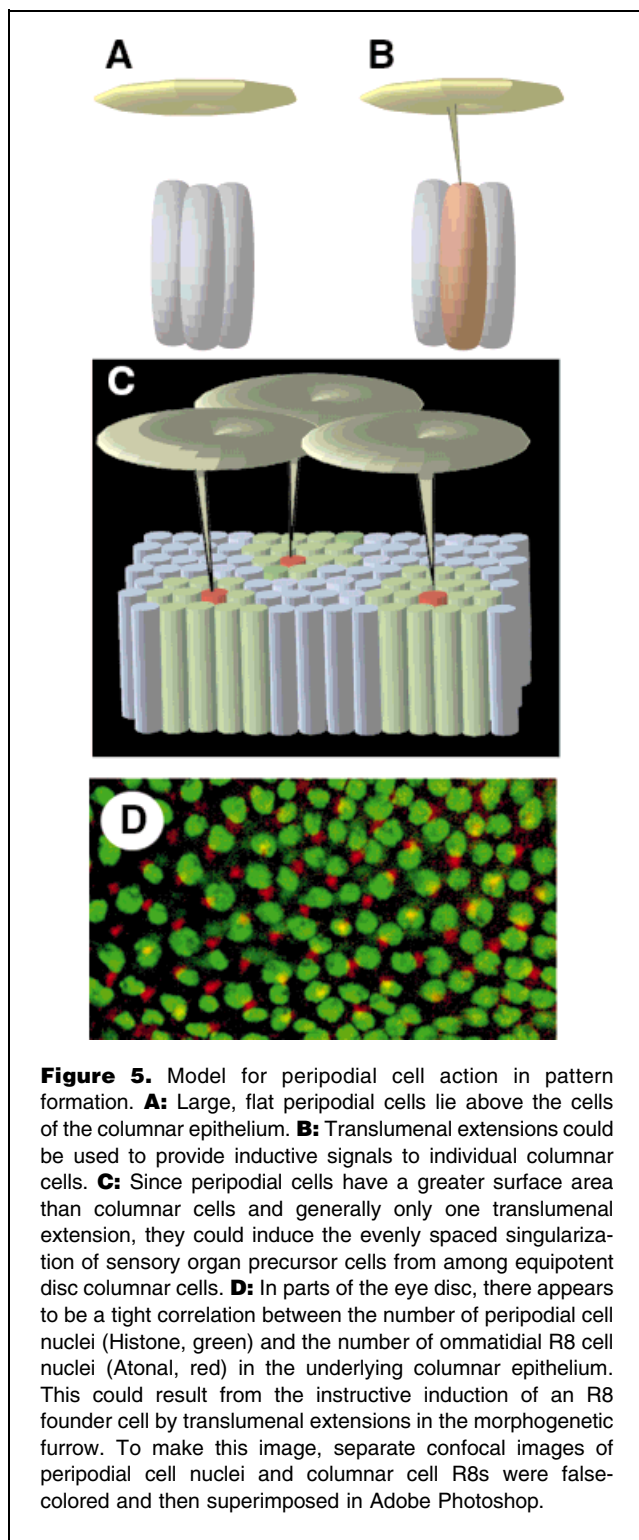


Figure 5. Model for peripodial cell action in pattern formation. **A:** Large, flat peripodial cells lie above the cells of the columnar epithelium. **B:** Translumenal extensions could be used to provide inductive signals to individual columnar cells. **C:** Since peripodial cells have a greater surface area than columnar cells and generally only one translumenal extension, they could induce the evenly spaced singularization of sensory organ precursor cells from among equipotent disc columnar cells. **D:** In parts of the eye disc, there appears to be a tight correlation between the number of peripodial cell nuclei (Histone, green) and the number of ommatidial R8 cell nuclei (Atonal, red) in the underlying columnar epithelium. This could result from the instructive induction of an R8 founder cell by translumenal extensions in the morphogenetic furrow. To make this image, separate confocal images of peripodial cell nuclei and columnar cell R8s were false-colored and then superimposed in Adobe Photoshop.

translumenal extensions over the morphogenetic furrow is approximately equal to the spacing of nascent ommatidia in the columnar epithelium. This approximate 1:1 ratio is further evidenced by a correlation between the number of peripodial

cells and the number of ommatidial clusters in some regions of the developing eye disc (Fig. 5D). One possible interpretation of this observation is that peripodial translumenal extensions define the position of R8 cells in the underlying columnar epithelium during furrow progression. In the case of macrochaete precursor cell specification in the wing disc, the extant model of lateral inhibition is insufficient to explain the apparently non-random singularization of sensory organ precursor cells from proneural clusters.^(46,47) Perhaps signals from peripodial translumenal extensions provide an inherent spatial bias for the specification of single sensory organ precursor cells in the columnar epithelium. Peripodial signaling could directly inform cell fate decisions, or, alternatively, influence the spatial arrangement of different cell types. We recognize that while such speculation can be quite interesting, only continued experimental analysis will help us understand the possible roles of peripodial cells in pattern formation.

Concluding remarks

We have discussed evidence that peripodial epithelia play important and unexpected roles in development of the *Drosophila* eye. It is interesting to note that *Drosophila* and its Dipteran kin most certainly evolved from an ancestor with a peripodial-less eye primordium, such as is observed in the Lepidopteran *Manduca sexta*. In the peripodial-less ancestral system, there is still growth, there is still a morphogenetic furrow, and indeed, there is still a compound eye.^(48,49) Why would *Drosophila* invent novel peripodial-dependent mechanisms to perform functions present in the ancestral system? One possibility is that the evolutionary transition from evaginated buds to invaginated sacs placed new demands on disc development while simultaneously creating the structural possibility for novel interactions between peripodial and columnar cells. Along these lines, *Drosophila* eye discs form early (during embryogenesis) and grow rapidly throughout larval development. In contrast, *Manduca's* peripodial-less eye primordium does not initiate proliferative growth until after the last larval instar.⁽⁵⁰⁾ Peripodial–columnar cell interactions might somehow account for the relatively early and aggressive developmental profile observed in *Drosophila* imaginal discs. Intriguingly, *Manduca* wing discs possess peripodial epithelia and are the only *Manduca* adult primordia to form during embryogenesis and grow during larval development. This is consistent with the idea that peripodial cells promote growth of discs during early stages of larval development. There are clearly alternative explanations for the evolutionary origin of peripodial–columnar cell interactions, so at present we can only conclude that this will prove a fascinating field for further inquiry.

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