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Principles of *Drosophila* Eye Differentiation

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Abstract

The *Drosophila* eye is one of nature's most beautiful structures and one of its most useful. It has emerged as a favored model for understanding the processes that direct cell fate specification, patterning, and morphogenesis. Though composed of thousands of cells, each fly eye is a simple repeating pattern of perhaps a dozen cell types arranged in a hexagonal array that optimizes coverage of the visual field. This simple structure combined with powerful genetic tools make the fly eye an ideal model to explore the relationships between local cell fate specification and global tissue patterning. In this chapter, I discuss the basic principles that have emerged from three decades of close study. We now understand at a useful level some of the basic principles of cell fate selection and the importance of local cell–cell communication.

We understand less of the processes by which signaling combines with morphogenesis and basic cell biology to create a correctly patterned neuroepithelium. Progress is being made on these fundamental issues, and in this chapter I discuss some of the principles that are beginning to emerge.

1. Introduction

The retina is a favorite tissue of developmental neurobiologists as it is a relatively simple neuroectoderm. Two major types of retinas have evolved. The *simple camera eye* is favored by larger animals including most vertebrates. It is composed of three components: a lens, a retina, and a pigment layer. The simple camera eye has the considerable virtue that it the more sensitive eye with higher resolving power. But it requires a minimum distance between lens and retina due to the refractive index of a biological lens. Smaller animals including most insects favor the *compound eye*. It also contains lens, retina, and pigment layer, but the three are compressed and compartmentalized to fit onto tiny heads. The two types of retinas share many of the molecular factors required to direct development as well as many of the channels, etc., required to permit function, as well as similar neuronal subtypes. They differ in their overall structure and the germ layers from which they emerge.

Understanding construction of epithelia—and neuroepithelia in particular—remains a central challenge. We will need to improve our understanding of how local features at the level of individual cells influences higher order tissue patterning. In this chapter, I examine the processes that shape the emerging *Drosophila* eye with an emphasis on tissue morphogenesis and patterning. I will outline basic findings and encourage the reader to follow the references provided for greater detail. Instead, I focus on providing an overview to place these issues in context, then discuss some basic principles that have emerged from these studies.

2. The Developing *Drosophila* Eye

The *Drosophila* eye has proven a powerful model for understanding how local cell–cell signaling activates signal transduction pathways to direct cell fate. The eye has provided several of the first examples of directed cell fate within an epithelium and, along the way, has helped us better understand how the Notch, RTK/Ras, Hedgehog, and Wnt pathways can work together

as cell fate “switches.” Recently, developmental biologists have sought to look deeper into the cell biology of emerging cell types to understand how cell intrinsic properties contribute to building an epithelium. Other systems such as *Drosophila* embryonic gastrulation are better suited to understanding large movements of cells across an epithelium. However, the retina remains a model of choice for understanding the smaller-scale movements of cells within an epithelium that are critical for its proper assembly. In this section, we will review work that looks (1) inward at the details of a cells' biology and how it affects overall eye field structure and (2) outward at the cell biology ‘rules’ that govern rearrangement of cells into useful patterns. This work represents a maturing of the fly eye field, further increasing its utility as one of the best understood developing epithelia.

2.1. The *Drosophila* adult eye

The *Drosophila* compound eye contains approximately 700 (male) to 750 (female) unit eyes known as ‘ommatidia’; the adult structure is presented in Fig. 5.1. Each ommatidium consists of a core of eight photoreceptor neurons, capped by four non-neuronal cone cells and two primary pigment cells that together form an ‘iris.’ These ommatidial cores are optically insulated from neighboring ommatidia by an interweaving hexagonal lattice of secondary and tertiary pigment cells that prevent light from passing between ommatidia. This arrangement is astonishingly precise, aiming each ommatidium in an exacting outward angle to evenly cover the visual field. Assembling this highly derived structure presents a challenge: the acuity of the fly's vision is directly related to the precision by which it can construct and place each ommatidium. Insects have solved this challenge through an evolving series of patterning choices that coordinate cell signaling, cell proliferation, cell death, and cell movements. The result is one of nature's more stunning structures and, from a developmental biologist's standpoint, one of its most useful.

2.2. Establishing the eye field

Clonal analysis indicated that the eye field is derived from approximately six cells that are set aside early in embryogenesis (Wieschaus and Gehring, 1976). These six cells give rise to a structure with several basic strengths that make it in many ways an ideal model system.

As with most other adult *Drosophila* structures, each eye emerges from an ‘imaginal disk’ (from *imago*, meaning adult insect), specifically the eye portion of the eye/antennal disk (‘eye disk’). This peninsula of tissue begins as an ectoderm-derived infolding that is originally established—and split into two eye fields—through the combined efforts of Decapentaplegic (Dpp) and Hedgehog (Hh) pathways (Chang *et al.*, 2001) in a manner analogous to signals that establish the vertebrate eye field (Yang, 2004). These signals then act with the Notch and EGF-receptor pathways to induce proliferation as well as a complex web of signals that establish and define the eye field (Fig. 5.2; Chang *et al.*, 2003; Kenyon *et al.*, 2003; Kumar and Moses, 2001). The emergence of the *Drosophila* eye from the ectoderm overlying the brain is more similar to the vertebrate lens than its retina; nevertheless, the two retinas show marked similarity in their molecular underpinnings (Chang *et al.*, 2001; Pichaud *et al.*, 2001). Infolding of the fly eye disk results in a two-layered tissue: the future eye field thickens into a pseudostratified epithelium that is covered by a thin squamous epithelium, the ‘peripodial membrane’. This eye anlage is easily plucked out of the organism and imaged as a whole mount, a useful property.

Six core transcription factors (*eye*, *toy*, *optix*, *so*, *eya*, *dac*) interact amongst themselves and other noncore factors to establish the eye field (Fig. 5.2). Each of these six factors is active in establishing the mammalian eye, and mutations can lead to serious diseases of the retina and elsewhere. In addition to establishing the eye field (i.e., conferring competence to a set of cells for response to eye-specific signals), these six factors are active in promoting cell proliferation

and cell fate by acting with numerous other factors in the nucleus and at the surface (reviewed in Kumar, 2008; Pappu and Mardon, 2004).

2.3. The morphogenetic furrow and emergence of the ommatidial core

The *Drosophila* life cycle includes three larval stages that together span four days. For most of larval life the eye disk proliferates, broadly establishing the eye field and dividing it into dorsal and ventral regions. Cell-type differentiation begins midway through the final larval stage. The first sign is emergence of the ‘morphogenetic furrow’, a physical indentation in the eye field that appears initially near the posterior edge of the eye/antennal disk. This furrow ‘sweeps’ anteriorly as progressive rows of cells utilize actin/myosin dynamics to alter their shapes and sink basally (Benlali *et al.*, 2000; Escudero *et al.*, 2007). Ahead (anterior to) the morphogenetic furrow, cells continue to proliferate and expand the eye field. The furrow itself is a point of cell cycle arrest as cells enter G1. Cell fate determination begins within the furrow and continues to progress behind (posterior) to it. Despite providing a striking demarcation in the emerging eye field—a sort of moving anterior/posterior boundary dependent on Hedgehog and Dpp signaling (Heberlein *et al.*, 1993; Ma *et al.*, 1993)—the function of the morphogenetic furrow is not known.

Our emerging understanding of the processes that direct cell fate determination represents a striking success of modern developmental biology; it has been well reviewed (e.g., Nagaraj and Banerjee, 2004; Voas and Rebay, 2004; Wernet and Desplan, 2004). In short, local signals are shared between cells that are ‘read’ as cell fate information. Therefore, the position of a cell within the eye field determines its fate. This process leads to progressive induction of cell fates beginning with photoreceptor neurons (first neuron R8, then R2 and R5, R3/R4, R1/R6, and finally R7 within each ommatidium). Four glial-like Semper or ‘cone cells’ are recruited to each photoreceptor octet and, in the young pupa, two additional glial-like ‘primary pigment cells’ (1°s) complete the 14-cell ommatidial core (Fig. 5.3). Finally, an interweaving lattice of secondary and tertiary pigment cells (2°s, 3°s) and sensory bristle organules emerge in the pupa to organize the ommatidial array into a precise pattern. The cone and pigment cells serve to secrete the overlying lens, turn over rhabdomere membrane, and limit the pathways of light permitted to enter each ommatidium.

While we understand quite a bit regarding the signals that direct particular cell fates, we understand very little about the spatial organization by which these cells collect together. For example, all cell types exempting R8 and R7 emerge in symmetric pairs in which each cell mirrors its partner's position across the growing core (some insects exhibit pairwise R7-like neurons). The presumption is that this symmetry reflects symmetric cell signaling within the growing core (Freeman, 1997; Tomlinson and Ready, 1987), but the factors that place R1/R6 on a side (initially) posterior to R3/R4 is not understood. Nor do we understand the mechanisms that limit the number of photoreceptor neurons specifically to eight in *Drosophila*. The *Drosophila* eye presents a uniquely simple model for studying these small-scale issues of tissue patterning and remodeling yet these issues remain a challenge to the field.

2.4. Signaling from the peripodial membrane

One of the truly surprising findings that have emerged from studying eye development is the direct regulation of cell fate by the overlying peripodial membrane. Recall that the peripodial lies as a thin squamous cap over the eye field. Two different groups (Cho *et al.*, 2000; Gibson and Schubiger, 2000) identified a remarkable physical process that emerges and extends from individual cells within the peripodial membrane to contact individual cells within the underlying eye field. This microtubule-dependent extension brings with it signal transduction molecules including Hedgehog, Dpp (a BMP ortholog), and Wingless (Wnt) that help regulate cell fate. This is a remarkable and nearly unprecedented example of individual cells from one

epithelium physically reaching across to another to direct development of individual target cells. If commonly utilized in development, the significance of this mechanism is large and holds the potential to endlessly complicate the study of cell fate induction.

2.5. Planar cell polarity

Another aspect of patterning and morphogenesis in the fly eye is planar cell polarity. Nearly all epithelia have an intrinsic, cell autonomous polarity. This polarity is reflected across the plane of the eye's surface as each ommatidium rotates, leaving photoreceptor R7 toward the dorsal/ventral equator at the eye disk's center (Fig. 5.3A). For each ommatidium to achieve its correct polarity the initially symmetric R3 and R4 pair must be distinguished, presumably to break the ommatidium's initial symmetry and provide it 'handedness'. Once established, each ommatidium then acts on this polarity by rotating 90° in a clockwise (if located within the dorsal half of the eye field) or counterclockwise (ventral) fashion. Several factors important in developing an R3/R4 distinction are known and, interestingly, these same factors mediate planar cell polarity in other animals (reviewed in Seifert and Mlodzik, 2007; Simons and Mlodzik, 2008). These include the antagonistic protein complexes Frizzled/Flamingo and Strabismus/Prickle that partition within a cell to provide autonomous polarity. In a mechanism that is not well understood, a signal involving noncanonical Wnt pathway activity provides spatial information to each R3/R4 pair across the eye field.

The precise mechanism that distinguishes R3 from R4 is not understood in part because no ligand has been identified capable of signaling across the eye field (e.g., none of the Wnt orthologs clearly play this role). Further looming, we understand very little about the cell biological processes that mediate the physical rotation of ommatidia that turn 90° in the span of several hours. Reducing the activity of one gene is known to affect specifically rotation: the serine–threonine kinase Nemo (Choi and Benzer, 1994; Fiehler and Wolff, 2008). Other factors mediating both surface adhesion and cytoskeletal rearrangement are likely to be centrally involved, but little data have surfaced for either of these components.

2.6. Fundamental principles I: *Drosophila* eye assembly

The eye is an elegantly simple structure. Its constituent ommatidia are organized into a precisely staggered pattern evolved to optimize coverage of the fly's visual field. The importance of this repeating pattern as a strength of the eye as a model system should not be underestimated: subtle changes are more easily and confidently observed when a field contains hundreds of repetitions. Through the concerted efforts of many laboratories we have learned many of the fundamental principles utilized to assemble a fly eye:

The eye matures in a stepwise fashion—The eye emerges in just a few days and is easily accessible both physically and genetically, permitting us to readily observe in detail many of the basic processes that assemble the eye. The eye first enlarges through proliferation before specific cell types emerge. General axes—D/V, A/P—are also set early and independent of cell-type specification. Finally, the animal monitors its eye carefully. Expression of, for example, oncogenes targeted to specifically disrupt the size and integrity of the eye field causes the animal to delay pupariation (Pedraza *et al.*, 2004; Read *et al.*, 2004), presumably to give the eye time to right itself.

Molecular pathways are utilized reiteratively—The same core signaling pathways are utilized multiple times throughout eye development, particularly the EGFR/Ras, Notch, Dpp (BMP), Wg (Wnt), and Hedgehog pathways. This phenomenon has been observed in most developing tissues, and studies in the fly eye have been central to our understanding of how a single pathway can direct development of so many cell decisions from fate to morphogenesis. A common motif is the dynamic progression of transcription factors and other molecular

mediators: general surface signals achieve specificity by altering (e.g., phosphorylating, degrading, etc.) evolving sets of targets. In the past, this changing cellular set of targets was somewhat mysteriously referred to as “competence.” While we have a deepening understanding of how this works in terms of evolving transcription factors, our understanding of evolving morphogenesis-related factors (e.g., regulators of junction or cytoskeleton dynamics) is less mature.

Position determines cell fate—A cell's position and its competence determines how it will act on a ‘generic’ surface signal. For example, a cell next to the R8 photoreceptor cell that receives a *Notch*-plus-*Ras* signal in the mature larva will develop as an R7 photoreceptor cell due to expression of factors such as *prospero* (Xu *et al.*, 2000). The same signal provided a day later by a cone cell can act on factors such as *dPax2* in its neighbor to direct a glial-like support cell fate (Flores *et al.*, 2000). Same signal, different response.

3. Patterning the *Drosophila* Eye: Morphogenesis and Cell Movements

In this section, we examine a more recent focus of the fly eye field. The remarkable simplicity and precise pattern of the eye is also a powerful advantage for examining the details of how cell–cell contacts and cell movements mediate tissue patterning. This task has been helped recently with the establishment of live imaging. The eye disk migrates to the surface of the animal early in pupation; opening the cuticle allows use of higher resolution microscopy, and junction-associated green fluorescent protein (GFP) nicely outlines the apical surface of cells. In this section, we focus on events in the pupal eye with an emphasis on the morphogenesis of pattern formation.

3.1. Differential adhesion patterns the cone cell quartet

Beginning in the late larval stages, the four cone cells within each ommatidium push through the photoreceptor cluster to generate a tight cap at the top of the retina. This cap will eventually serve as the ommatidium's ‘iris.’ The apical profile of the four-cell cluster is approximately ellipsoid (Fig. 5.3). Hayashi and Carthew (2004) pointed out that this configuration is predicted by “soap bubble”-based models that focus on differences in relative adhesions. They demonstrated that this cone cell quartet has strongly selective preference for adherence amongst themselves based on their selective expression of N-cadherin. Neighboring 1°s do not express N-cadherin, effectively segregating the cone cells and encouraging them to collect into a predictably shaped cluster. Loss of N-cadherin led to a more elongate “cruciform” shape of the cone cell quartet; additional removal of E-cadherin led to still more dramatic shape changes (Hayashi and Carthew, 2004).

This work provides an excellent example of understanding local cell arrangements based on differential adhesion. Utilizing this concept allowed Hayashi and Carthew to predict the final patterns of various numbers of cone cell groups—altered through gene mutation—based on the principle of most efficient packing (Fig. 5.5); indeed, the precise angle of cell contacts was successfully modeled mathematically (Hilgenfeldt *et al.*, 2008; Kafer *et al.*, 2007). Applying the concept of minimization of free energy to cell packing was first proposed by Steinberg (Steinberg, 1970; Steinberg and Poole, 1981). In Steinberg's “Differential Adhesion” model, collections of cells have a natural drive to sort into configurations that minimize the system's free energy: for example, cells with high levels of the homophilic adhesive protein E-cadherin will naturally sort together at the core of a cell collection, pushing cells with lower “adhesiveness” to the periphery. More specifically, cone cells minimize the system's free energy by decreasing surface contacts with 1°s, a drive that—paired with “elastic tension” to promote stable forms—encourages rounding of the cone cell quartet's outer profile. Patterning of the cone cells presents an elegant example of this process in the context of two-dimensional sorting of their apical profiles. Next, I consider an alternative strategy, increasing cell contacts

between different cells, that nevertheless heeds the same taskmaster of decreasing overall free energy.

3.2. Emergence of the interommatidial lattice

My laboratory has focused on the events that rearrange interommatidial cells into a remarkably precise hexagonal lattice of 2°s, 3°s, and bristle organules that weave through and organize the ommatidial array. This process is a useful model for long-range patterning and re-organization across an epithelium and has even been modeled computationally (D. Larson *et al.*, unpublished data). Initially, ‘interommatidial precursor cells’ (IPCs)—precursors of the 2°s and 3°s—lie unpatterned between the ommatidial cores (Fig. 5.3E). Within a few hours, (1) the IPCs line up in single file while (2) some of the IPCs are removed to get to the final required cell number. When the process is complete, each 2° is stretched between two 1°s and each 3° (and each bristle, sort of) sits between three 1°s (Fig. 5.3G). This process aligns ommatidia into a staggered array that best covers the visual field. While organizing the ommatidial lattice should improve visual precision this organization is not the rule in compound eyes: most arthropoda leave their pigment cell lattice poorly organized (e.g., Fig. 5.3H), emphasizing the derived nature of this patterning process. Nevertheless, some familiar factors mediate its progression.

Current work on IPC patterning is beginning to link signal transduction with adhesion and cell biology. Four signaling pathways play a primary role in 2°/3° maturation and assembly: Notch, EGFR, Wg, and Dpp (BMP-like) activities combine to regulate cell fate, cell positioning, and cell death; disruption of any of these pathways leads to incorrect patterning (Cagan and Ready, 1989a; Cordero *et al.*, 2004, 2007; Freeman, 1996; Reiter *et al.*, 1996; Yu *et al.*, 2002). Their roles are not fully understood but, roughly speaking, EGFR primarily regulates all cell fates, Wg regulates cone cell fate (which affects later patterning; J. Cordero and R. Cagan, unpublished data) and cell death, and Dpp regulates cell positioning and a correctly formed apical profile. Notch has the most severe effects and regulates several aspects including cell fate, cell death, and cell adhesion/assembly. Recent data suggest that Notch acts in part through regulating Hibris (Hbs) and Roughest (Rst) (S. Bao and R. Cagan, unpublished data). Hbs and Rst are members of the Nephrin superfamily (Nephrin and Nephl, respectively) of adhesion-like transmembrane proteins that show heterophilic adhesion to each other (Ramos *et al.*, 1993). Loss of either leads to incorrect IPC patterning due to a failure of IPCs to correctly move into and establish their required positions (Bao and Cagan, 2005; Reiter *et al.*, 1996). Two other Nephrin superfamily members, Sns and Kirre, play a somewhat redundant role in this process (S. Bao and R. Cagan, unpublished data).

This reliance on adhesion-like factors has led to a “Preferential Adhesion” model in which the epithelium has a drive toward minimizing the system's overall free energy by maximizing contacts between 2°/3°s and 1°s. This model is yet another twist on Steinberg's Differential Adhesion model: by utilizing two heterophilic adhesion proteins, potential pattern complexity greatly increases from simple soap-bubble-like collections of cells to complex patterns of ommatidia woven together by a separate hexagonal pigment cell lattice. However, more is to come on this process. Nephrin family members—including Hbs, Rst, Sns, and Kirre—can directly signal into cells and recent work suggests they provide a link between the surface and rearrangement of the cytoskeleton (Johnson *et al.*, 2008; Seppa *et al.*, 2008). This rearrangement would presumably mediate cell movements during the patterning process. Recent work suggests that control of the apical profile, for example, through nuclear movements (as the nucleus rises toward the surface the apical profile increases to accommodate its size) plays an important role in patterning (D. Larson, R. Johnson, M. Swat, J. Cordero, J. Glazier, and R. Cagan, unpublished data).

3.3. Fundamental principles II: Long-range patterning

Cells are placed in their correct positions using basic *patterning rules*. So what have we learned so far from work on patterning events in the pupal eye? A few points are worth mentioning, although clearly this is a work in progress:

The eye epithelium steadily calibrates cell number—To achieve a precise pattern, the eye field likely must keep the number of cells between ommatidial pattern elements within a certain range. Too many IPCs will be difficult to efficiently remove, and indeed the adult eye does contain a few ectopic 2°s (which share a single 2° niche and do not disrupt the overall pattern). Too few IPCs will result in direct contact between neighboring ommatidia and the potential for optical bleed-through.

To calibrate IPC number the eye uses a steady combination of cell cycle and cell death. Regarding the former, after establishment of the initial ommatidial ‘precluster’ (assembly of photoreceptor neurons R2–R5 and R8) the remaining cells undergo a final ‘second wave’ of proliferation to provide additional cells between nascent ommatidia (Ready *et al.*, 1976). Levels of proliferation are calibrated through Notch and EGFR pathway activities that, in turn, spatially control proliferation through signaling from the ommatidia themselves (Baker, 2001; Baonza and Freeman, 2005; Firth and Baker, 2005).

The second method of regulating cell number is through programmed cell death. Excess cells are cued to die by classic apoptosis (Cagan and Ready, 1989b; Wolff and Ready, 1991). Two mechanisms are known to regulate the position and number of programmed cell deaths. In the larva, competition for ommatidial-provided EGFR ligand leads to the death of cells located far from ommatidia (Baker, 2001; Yang and Baker, 2003). In the pupal eye, IPC patterning requires removal of cells simultaneous to their rearrangement (Cagan and Ready, 1989b; Wolff and Ready, 1991). Death is preceded by activation of the classical *Drosophila* apoptotic pathway, which leads to activation of multiple caspases and subsequent apoptosis (reviewed in Brachmann and Cagan, 2003). Cell death in the pupal eye appears to be a competition between cells to maximize contact with the neighboring 1°s, but the precise nature of the mechanisms that determine which cell lives and which dies within a niche is not understood.

Patterning begins at the apical surface—Based on tissue reconstruction from electron micrographs, contacts between cells in the emerging eye occur first at the apical surface. This has been most clearly catalogued in the pupal eye (Cagan and Ready, 1989b), where cells make striking changes across the epithelium. Typically as a cell moves within the epithelial field, it (1) extends a small ‘process’ to contact a cell 1–2 cell diameters away, (2) moves its apical surface to establish a contact and junction with that cell, and (3) the contact is ‘zippered down’ basally to move the remainder of the cell into the new position (Fig. 5.4; Cagan and Ready, 1989b). This ‘apical first’ style of cell movement simplifies the patterning process by reducing it to a two-dimensional problem. Most important signaling and junction-related proteins are found at or apical to the apical adherens junctions. Again, collecting these factors at the surface aids in simplifying the signals and adhesions needed to correctly move and pattern cells.

Some questions still remain regarding the role of the adherens junctions themselves in the patterning process. As discussed below, cadherins are important for patterning cone cells and altering them leads to a poorly patterned quartet. Adherens junctions—as assessed by the presence of cadherins—do show some dynamic properties but for the most part there is not a clean correlation between absence of adherens junctions and cell movements. Movies that visualize cell movements in the live eye *in situ* show that cells with apparently significant junctions still freely exchange contacts and move to other sites (Larson *et al.*, 2008). Either junction strength is regulated in a manner other than by regulating the presence of Cadherin at the cells' surface (perhaps it is regulated by cytoplasmic proteins that link Cadherins to the

cytoskeleton), adherens junctions are more easily broken than expected, or other factors override a cell's junction with its neighbor.

Events are integrated—Work on patterning of both the cone cells and the pigment cells have emphasized the importance of cell–cell adhesion. However, we know that both Cadherin and Nephrin family members also signal through interactions with cytoplasmic proteins. Mutations in some of these proteins—for example, orthologs of CD2AP (Cindr) and ZO-1 (Pyd)—show patterning defects (Fig. 5.5E; Johnson *et al.*, 2008; Seppa *et al.*, 2008), emphasizing this point. The most likely result of these sorts of intracellular signals is modification of the actin cytoskeleton. Indeed, morphogenetic events in the larval eye (Corrigall *et al.*, 2007; Escudero *et al.*, 2007; Schlichting and Dahmann, 2008) as well as the pupal eye (Johnson *et al.*, 2008; Seppa *et al.*, 2008) require precise actin remodeling as cells execute fine movements. We appreciate that dynamic regulation of cells' cytoskeleton is a central component to regulating morphogenetic furrow formation, ommatidial rotation, and selective adhesion. However, we only poorly understand how signals and adhesion are translated into re-organization of the cytoskeleton and subsequently back to the surface to affect cell movements. Nor do we understand how this process is regulated spatially to move cells into their proper niche or rotate ommatidia in the proper direction. The connection between long-range signaling, short-range cell–cell interactions, and actin remodeling holds perhaps the greatest potential for surprises over the next few years.

More complex patterns require more surface factors—Cone cells are an elegant and simple system that, at their core, appear to require a single predominant adhesive factor: N-Cadherin. This simplicity is reflected in four-cell packing that cleanly reflects the dynamics of soap bubbles (Hayashi and Carthew, 2004). Introducing two factors that interact in heterophilic adhesion dramatically increases the potential complexity of the system, and IPC patterning provides an example. In addition to adding an extra adhesion factor, IPC patterning also requires precise, dynamic expression of each of the factors: Hbs (and Sns) must be expressed in the cone cells and 1°s at precisely the correct stage, and Rst (and Kirre) must be expressed in a complementary fashion in the IPCs. To accomplish this, regulation comes at the level of both controlled expression and protein turnover (S. Bao and R. Cagan, unpublished data). This in turn opens several points of potential regulation to achieve still greater complexity and nuance. Therefore, the progression of patterning in the pupal eye permits us to study increasingly complex epithelial patterning mechanisms, a useful training exercise for grasping the still greater complexity of the maturing mammalian nervous system.

Cells maximize their contacts with preferred neighbors—Many of the mechanisms by which cells are assembled into precise patterns can be inferred simply by looking carefully at their contacts during the patterning process. Apical IPC cell profiles change from smooth to ‘scalloped’, effectively increasing their length of contact with neighboring 1°s (Fig. 5.5C). By contrast, IPCs do not exhibit ‘scalloped’ contacts with other IPCs (Fig. 5.5C) indicating low IPC–IPC adhesion.

The pigment cell pattern does not count cells—The cone cell quartet is a small, limited pattern element that depends heavily on the number of cells. By contrast, the “Preferential Adhesion” model makes the surprising prediction that heterophilic adhesion drives IPC patterning independent of cell number. We see this prediction verified in wild-type eyes. The normal adult hexagonal array of ommatidia is precise even though it contains many ectopic 2°s that are not removed during development. These ectopic 2°s do not affect the overall pattern, but rather two 2°s can share a hexagonal face without distorting the pattern by reducing their apical profile. Reducing cell death produces still more ectopic 2°s, again with minimal affect on the overall pattern (Fig. 5.5F).

How can an epithelium create a precisely patterned structure with little regard for cell number? The answer can be deduced when Rst—the adhesion factor expressed in IPCs—is strongly over-expressed well above its normal levels in single isolated cells. These cells expand their apical profiles and take over a 2° *plus* a 3° niche (Fig. 5.5G). Apparently the patterning system does not count cells but rather expands membranes to equilibrate and maximize Rst/Hbs-mediated contacts. When the concentration of the adhesion molecule is out of balance with the number of cells, adhesion wins.

Nuclear movements—The movement of a cell's nucleus also affects surface patterning. Cells in the eye are very elongate and thin: each larval cell is perhaps 30–40 μm long and just a few microns in width. The exception is the nucleus, which is often more than 10 μm in width and is less flexible than the cell body (schematized in Fig. 5.3B). As a result, when a cell's nucleus is basal the cell's apical profile tends to be thin; as the nucleus rises toward the surface during differentiation and patterning the apical surface can more than double in a few hours. This change dramatically alters the patterning landscape. For example, the nuclear movement-dependent expansion of ommatidia's apical profiles help 'push' neighboring IPCs into hexagonal patterning (D. Larson *et al.*, unpublished data).

4. Conclusion

The role of nuclear positioning is an interesting example of how intrinsic cell biological properties can influence a cell's positioning in surprising ways. It is also a reminder that we have some work to do to be able to answer the deceptively simple question: how is an epithelium patterned? Since publication of the seminal work by Ready, Hansen, and Benzer three decades ago (Ready *et al.*, 1976), the field has shown remarkable success by selecting very specific questions to address: cell fate induction, morphogenetic furrow progression, cell movements and patterning, etc. As a result, we view events as occurring along a strict timeline in which each step directs the next as neighbors collide and signal locally.

As we expand our understanding of specific aspects of cell biology that direct cell morphogenesis and epithelial patterning, this view will change. Cells are capable of reaching 'down' from overlying peripodial membrane to signal the eye disk (Cho *et al.*, 2000; Gibson and Schubiger, 2000) and across developmental time and space as cells within the morphogenetic furrow influence planar cell polarity by extending Scabrous-containing processes across the eye field (Chou and Chien, 2002). The outcome of eye morphogenesis and patterning is appealing in its simplicity. But the developmental processes required to build a fly eye are sufficiently complex to provide useful models for many of the basic aspects of epithelial maturation and, eventually, a more integrated view of the process.

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Figure 5.1.

The *Drosophila* adult compound eye. The adult *Drosophila* eye is a model of precision. Anterior is to the right. (A) The adult compound eye is composed of more than 700 precisely arranged ommatidia. This precision combines with the eye's curvature to evenly cover the fly's visual field. (B) Section through an adult eye approximately 30 μm below the eye's surface, showing a field of 10 whole ommatidia. The seven apical photoreceptor neurons of one ommatidium are labeled within the cell bodies; their (blue) rhabdomeres are arranged in a trapezoid. Note how the precise hexagonal lattice of red pigmented 2°/3°s between ommatidia (darkened by methylene blue staining) optically insulate each ommatidium. (C) Schematic of a single ommatidium drawn to scale. The third "section" matches panel B (from Cagan and Ready, 1989b).

Figure 5.2.

Establishing the eye field. (A) Comparing emergence of vertebrate and *Drosophila* eyes during early neurogenesis. The *Drosophila* eye remains as part of the surface ectoderm (from Chang *et al.*, 2001). (B) The network of factors known to establish the eye field (adapted from Kumar, 2008).

Figure 5.3.

Larval and pupal eye development. Ommatidial differentiation and eye pattern formation begin in the third instar larva (panels A–D) and continue through early pupal stages (panels E–G). Anterior is to the right. (A) Photoreceptors emerge progressively and in symmetric pairs: R8, R2/R5, R3/R4, R1/R6, and finally R7 emerge stepwise and symmetrically across each ommatidium's central axis. Planar cell polarity (PCP) is revealed by progressive rotation of each ommatidium (yellow arrows). Distinguishing photoreceptors R3 and R4 (*) within each ommatidium is a necessary step for establishing its polarity. (B) In the pupal eye, the apical portions of the cone cells and pigment cells (1°, 2°, 3°) push over the photoreceptors to dominate the surface; the photoreceptor octet has been pushed below the surface to lie just

below the cone cells and 1° s (*). The Z-axis is provided to indicate that, while cell movements initiate at the apical surface, each cell extends to the basement membrane. The area of each cell's apical profile is determined primarily by the proximity of its nucleus to the surface. (C) Breakaway schematic view of a larval photoreceptor cluster (from Kramer *et al.*, 1991). (D) A portion of a developing larval eye disk viewed at its apical surface. Cell membranes are highlighted with a cobalt sulfide stain. The morphogenetic furrow (MF) and the progression of photoreceptor neurons (1–8) and cone cells (c) are labeled. Arrows emphasize progression rotation of ommatidia. Compare with panel A. (E–G) Three views of a pupal eye field at 20, 24, and 42 h APF, respectively. IPCs are pseudocolored green. Initially unpatterned (panel D), IPCs rapidly re-arrange into single file as excess IPCs are removed by programmed cell death (panel E). Panel F shows the final pattern (compare to panel B). (H) Many compound eyes do not re-organize their interommatidial pigment cells to achieve tight patterning of the ommatidial array. Shown is a view of a mature cockroach eye with a region of six loosely organized 2° -like cells emphasized by pseudocoloring. Cone cells from an ommatidium (cc) are indicated. The adult ommatidial array remains poorly aligned (from Nowel, 1980).

Figure 5.4.

Cells make new contacts initially at the apical surface. A recently established 3° is pseudocolored in green. Electron micrographs (from Cagan and Ready, 1989b). (A) At the apical surface of the emerging pupal eye, the 3° has contacted 1°s from three different ommatidia. Arrows indicate the extent of the 3°s contact with one of the 1°s, which is pseudocolored in brown. (B) One micron deep, the 3°/1° contact is smaller (arrows). (C) A deeper view demonstrates how the contact has yet to extend basally; two neighboring IPCs occlude the 3° and likely had originally ‘competed’ to become the niche’s single 3°. The emergent 3° will rapidly extend its 1° contact basally through this region and to the retina’s basal ‘floor.’

Figure 5.5.

Adhesion directs assembly of cone cells and pigment cells. (A) Cone cell assemblies reflect the adhesive properties observed in adherent soap bubbles (from Hayashi and Carthew, 2004). (B) Loss of N-cadherin led to a “cruciform” shape (*left* panel) in which the long axis of the cone assembly is elongated (Hayashi and Carthew, 2004). Compare to the more rounded shape seen in panel A. Separation of cone cells also occurred (*right* panel), a predicted outcome of a cone cell’s decreased affinity for its companions. (C) Ommatidium from a 30-h-old pupa (Bao and Cagan, 2005). IPCs are pseudocolored green. Contacts between IPCs and 1°s are ‘puckered’ (e.g., arrows) to maximize 1°/IPC cell–cell contacts relative to IPC/IPC contacts. (D) Reduced *rst* activity led to poor assembly of the IPC lattice (Bao and Cagan, 2005). IPCs are pseudo-colored green. (E) Reducing Cindr, an adaptor protein known to mediate events between the surface and the cytoskeleton, led to abnormal IPC patterning (from Johnson *et al.*, 2008). (F) Mutations in the ubiquitin conjugase *morgue* partially blocked programmed cell death but did not alter the overall hexagonal pattern. Examples of ectopic 2°s pseudocolored in red (from Hays *et al.*, 2002). (G) Overexpressing Rst in individual IPCs led to a single cell (green) taking over two niches. Compare with the normal 2°–3° pair (purple in schematic inset) (from Bao and Cagan, 2005).