The genesis of retinal architecture: An emerging role for mechanical interactions?

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Abstract

Patterns in nature have always fascinated human beings. They convey the idea of order, organization and optimization, and, to the enquiring mind, the alluring promise that understanding their building rules may uncover the forces that shaped them.

In the retina, two patterns are outstanding: the stacking of cells in layers and, within the layers, the prevalent arrangement of neurons of the same type in orderly arrays, often referred to as mosaics for the crystalline-like order that some can display. Layers and mosaics have been essential keys to our present understanding of retinal circuitual organization and function. Now, they may also be a precious guide in our exploration of how the retina is built.

Here, we will review studies addressing the mechanisms controlling the formation of retinal mosaics and layers, illustrating common themes and unsolved problems. Among the intricacies of the building process, a world of physical forces is making its appearance. Cells are extremely complex to model as “physical entities”, and many aspects of cell mechanotransduction are still obscure. Yet, recent experiments, focusing on the mechanical aspects of growth and differentiation, suggest that adopting this viewpoint will open new ways of understanding retinal formation and novel possibilities to approach retinal pathologies and repair.

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\textit{Abbreviations:} AB, apico-basal; AJ, adherent junction; Crb, Crumb; DAH, differential adhesion hypothesis; DG, dystroglycan; ECM, extracellular matrix; GCL, ganglion cell layer; GFP, green fluorescent protein; ILM, inner limiting membrane; INL, inner nuclear layer; IOP, intraocular pressure; IPL, inner plexiform layer; NBL, neuroblastic layer; OLM, outer limiting membrane; ONL, outer nuclear layer; OPL, outer plexiform layer; RPE, retinal pigment epithelium.

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1. Introduction

The vertebrate retina is an outstanding example of modular neural circuitry. Its neurons are stacked in layers, separated by plexiform strata where processes and connections are confined (Fig. 1). Different cellular layers contain different neurons, and, within each layer, most neuronal types are arranged in regular arrays, known as retinal mosaics.

This modular organization has been the key to our current understanding of retinal function and organization. The stratification of neurons and processes in the mature retina has allowed the elucidation of a basic “functional unit” comprised of photoreceptors, bipolar, and ganglion cells, which, in each point in the visual field, operates from the capture of light to the delivery of visual information to the brain. The serial organization of this basic unit is directly reflected in the sequential position of its cellular components across the retinal depth, while the parallel arrangements of many such units allows visual processing throughout the visual field. Further exploration has uncovered the integrating mechanisms involving horizontal and amacrine cells, the different circuitry for rods and cones, and the ON and OFF pathways—to mention a few—all of which are wired in modular sub-circuits within the retina (Wässle and Boycott, 1991). Therefore, very schematically, the retina can be viewed as a tri-dimensional array of like functional units. Layers and mosaics are two main orthogonal views of such tri-dimensional organization (Fig. 1).

This review looks at retinal development through the lens provided by the studies investigating mosaic and layer formation. It is organized in four sections. The first section is a brief overview of the major steps in retinal development. The second section deals with retinal mosaics, their potential blue-print role in retinal development and our current knowledge of the mechanisms controlling their formation. The third section reviews studies addressing the molecular and cellular players that control layer formation in the retina. As it will be seen, many studies reveal an important role of adhesion molecules and their binding partners in controlling retinal patterning. This suggests that mechanical forces may have a significant role in retinal development or, for the sceptical, that a mechanical view to retinal development might provide interesting novel insights. For this reason, the last section will consider what we know about the mechanics of retinal development, alongside recent challenging data revealing unexpected effects of mechanical forces on cell growth and differentiation. This last section is inevitably highly speculative, but it is intended to illustrate new ways of approaching retinal formation, and to highlight their potential in dealing with retinal pathologies and repair.

2. An outline of retinal development

This section sketches an outline of vertebrate retinal development to be used as a reference frame for the
experiments described in the following sections. Data from multiple species are referred to, trying to draw a general common picture, and ignoring species-specific peculiarities. A more detailed presentation of retinal development can be found elsewhere (Sernagor et al., 2006).

2.1. A single proliferating cell layer

The eye develops from a lateral out-pouching of the neural tube which, through a sequence of inductive events, grows in size, folds inwards and develops into a cup formed by two cell monolayers (Fig. 2), the retinal pigment epithelium (RPE) in the outside, and the retinal neuroepithelium in the inside. The two layers are initially separated by a ventricle. In a first phase, both epithelia are proliferating, which leads to a rapid increase in the size of the cup. At this stage, the retinal neuroepithelium is an array of parallel-elongated cells, the neuroblasts, densely packed one next to the other. On the basal side, the cells are anchored to a basal lamina of extracellular matrix (ECM), the inner limiting membrane (ILM). At the opposite, apical side, which faces the RPE, the cells are “stitched” to one another by transmembrane protein complexes, the adherens junctions (AJs), which link the cytoskeletons of adjacent cells (reviewed in Tepass and Harris, 2007).

While neuroblasts are actively incorporating DNA and dividing, their nuclei perform a sort of back and forth dance, which brings the nucleus to a most basal position when DNA duplication is complete, and back to the apical region to engage in mitotic division (reviewed in Baye and Link, 2007). Throughout the cell cycle, cells retain their apical and basal links. Electron microscopy shows that dividing cells are linked through AJs to the adjacent cells (Kuwabara and Weidman, 1974). Real-time imaging (Fig. 3) shows that after a cell division, one of the daughter cells maintains the basal process of the progenitor, while the second daughter rapidly develops a new one (Das et al., 2003).
2.2. Two layers

The first cells to leave the mitotic cycle are the retinal ganglion cells (RGCs). Real-time imaging shows that a newly born RGC develops a growth cone from its basal process and from this an axon, which runs along the ILM, toward the optic nerve (Fig. 4). In the meantime, the RGC cell body is moved to the basal side of the retina, as if towed by the leading growth cone, and the apical process is retracted (Poggi et al., 2005a; Zolessi et al., 2006). Later, the cell starts to develop dynamic processes from the apical portion of the cell body, from which the dendritic tree develops (Kay et al., 2004). The basal migration of RGCs creates the first cellular stratification in the retina, which becomes subdivided into a neuroblastic layer (NBL) and a ganglion cell layer (GCL).
2.3. Four layers and at least four mosaics

Soon after the RGCs, horizontal cells, cones, and amacrine cells start being generated (Rapaport, 2006). All have migratory behaviours different from the RGCs. Both the cones and the horizontal cells remain in the NBL. The latter cells, which can be discriminated morphologically from the other NBL cells, have processes directed to the apical side of the retina (Ramon y Cajal, 1892, 1929), and cell bodies that move across the depth of the NBL (Edqvist and Hallbook, 2004). The amacrine cells migrate basally, towards the GCL, apparently retaining neither the apical nor the basal process (Fig. 5). Rather, they produce short dynamic processes in all directions, as if exploring the neighbourhood. Once they get close to the GCL, they stop the cell body migration and continue to “explore” the environment in all directions with short dynamic processes. The processes that run towards the GCL are selectively stabilized, grow longer, ramify, and eventually give rise to the cell dendritic tree. The thin layer of processes containing the amacrine cells dendritic trees, found at the basal side of the NBL, and apical to the GCL, is the first rudiment of the inner plexiform layer (IPL). Interestingly, the dendritic trees of amacrine cells are immediately aimed at either the apical or basal region of the IPL, where they are targeted by the RGC dendrites (Godinho et al., 2005).

At this stage, the retina has four morphologically distinguishable layers—the NBL, a rudiment of inner nuclear layer (INL) basal to and continuous with the NBL, the IPL and the GCL—and at least four detectable cell mosaics (Fig. 6)—a regular distribution of cholinergic amacrine cells in the GCL, a second one in the INL rudiment (Galli-Resta et al., 1997), and, in the NBL, an orderly subpopulation of cones (Bruhn and Cepko, 1996; Bumsted et al., 1997; Larison and Bremiller, 1990; Raymond et al., 1995; Wikler and Rakic, 1991, 1997), and a non-random array of horizontal cells (Novelli et al., 2007; Raven et al., 2005; Scheibe et al., 1995).

2.4. The mature pattern

The next step, in terms of strata, is the appearance of the outer plexiform layer (OPL), which is initially composed by the processes (and the first synaptic contacts) of the horizontal and the photoreceptor cells. At this stage, the retina is still missing its central “ingredient”, the bipolar cells, somewhat like a sandwich without part of its filling. In the last phase of neurogenesis, mostly rods, bipolar and Muller cells are born (Rapaport, 2006). The bipolar cells differentiate from neuroepithelial-like cells that develop short lateral processes from the basal process first, then from the apical process. Of these, the processes

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Fig. 5. Real-time imaging of amacrine cell migration: (a) Migrating amacrine cells show undirected neurite outgrowth. Time-lapse images of a YFP+ amacrine cell (arrow) migrating towards the GCL. Multiple neurites emerge from the cell, but they do not appear to be polarized towards the GCL. The dashed line represents the position of the future IPL, at the interface between the forming INL and GCL. (b) Amacrine cells display an early bias for the IPL sublamina in which they will ultimately stratify. Confocal time-lapse images of a pair of GFP+ amacrine cells (green). The red indicates the IPL. From the outset, either cell restricts dendritic ramification to either the basal or the apical sublamina of the IPL. Reproduced with permission from Godinho et al. (2005).
growing in the IPL and OPL become selectively stabilized and develop into axon and dendrites, respectively, while the main apical and basal processes of the cells are retracted (Morgan et al., 2006).

Very schematically, the development of the retina now requires only a few additional steps: the maturation of the photoreceptors, which form their “light detecting units”, the inner and outer segments; the lateral migration and compaction of central photoreceptors to form the fovea in primates; and the maturation and refinement of synaptic connections between the neurons. These processes are treated in details elsewhere (Usukura and Shuichi, 1996; Curcio et al., 1990; Wong, 1999).

3. The development of retinal mosaics

3.1. Early retinal mosaics and why we care about them

As we have seen, the retina is initially a tissue made by apico-basal (AB)-oriented elements that gradually converts into a structure characterized by cellular patterns—the layers and the cell mosaics—which are orthogonal to the AB axis. This patterning is progressively laid down by a “construction work” that goes on simultaneously within the different layers.

Some retinal mosaics are among the earliest detectable mature-like “patterned units” assembled during this building process. For this reason, we will start exploring the logic of retinal construction by reviewing what we currently know about mosaic formation. We will first consider which spatial (geometrical) rules underlie the regular spacing of cells within mosaics, then analyse studies addressing the potential contribution of cell genesis, death and migration to mosaic formation, and end up with a speculation on the nature of the cell interactions that underlay the assembly of cell mosaics.

3.2. The spatial rule that we call mosaic regularity

Retinal mosaics gained their name from the almost crystalline regularity of some cone arrays. Other cell types do not tile the retina as regularly, yet they still appear orderly when selectively stained in retinal whole-mounts. Since the seminal study by Wässle and Riemann (1978), a number of ways have been devised to explore the geometry of retinal mosaics. Essentially, they all show that mosaics are non-random cell distributions. Furthermore, modelling
and statistical analysis have shown that, in most cases, the geometrical rule underlying the non-random spacing of like-cells within their layer is a “minimal spacing rule” by which cells of the same type avoid being closer to one another than a given distance. This minimal distance is usually comparable to several cell body diameters, and varies with cell type (reviewed in Cook and Chalupa, 2000; Galli-Resta, 2002). The model of mosaic formation based on the implementation of a simple spacing rule has generated many interesting experiments, as it suggests that, in taking position within their layer, cells only care for similar (homotypic) cells, disregard cells of other types.

3.3. Predominance of homotypic-cell interactions in mosaic formation

A number of studies support the view that each cell type forming a mosaic does so mostly independently of cells of different types. In the adult retina mosaics formed by different cells types are not spatially correlated (Rockhill et al., 2000). Furthermore, experiments where the populations of pre- or postsynaptic partners are either ablated or genetically incremented do not affect mosaic formation in the case of the horizontal cells (Raven et al., 2007; Raven and Reese, 2003; Reese et al., 2005) or the cholinergic amacrine cells (Galli-Resta, 2000).

3.4. Potential players contributing to cell spacing within mosaics

As it is commonly observed in biology, there seems to be no single mechanism controlling cell spacing within retinal mosaics, but rather multiple processes appear to contribute to its development. In particular, investigations have been focused on cell death, cell fate control, and cell displacement.

3.4.1. Cell death in mosaic formation

Cell death has been proposed to contribute to the regularity of retinal mosaics on the basis of the following observations: (1) The mosaics of developing β-RGCs in the cat increase their regularity after undergoing a 20% cell loss (Jeyarasasingam et al., 1998). (2) In the bcl-2 over-expressing mice, some cell populations are increased as a result of the death sparing action of the transgene; in particular, the dopaminergic amacrine cells are about 10 times as numerous as in normal wild-type animals and display a more disorganized spatial arrangement than normal (Raven et al., 2003). (3) The minimal spacing between neighbouring cholinergic cells is decreased when cell death in this population is prevented by degradation of extracellular ATP or by blockade of the purinergic P2X receptor for ATP in the neonatal rat (Resta et al., 2005). (4) Finally, time-lapse analysis of the development of green fluorescent protein (GFP) labelled amacrine cells in the zebrafish, shows the frequent occurrence of cell death, with a higher likelihood to die for amacrine cells that come close to one another, in line with the view that death might contribute to like-cell spacing during development (reviewed in Poggi et al., 2005b).

While these experiments strongly suggest a role for death in mosaic formation, it is important to consider that cell elimination can improve the regularity of a distribution of cells only by removing wrongly positioned cells and not by correctly positioning cells. Thus, death should not be expected to create a regular distribution, unless it is complemented by “positive mechanisms”, such as continual cell addition and/or cell displacement. Two “positive” processes have been proposed to contribute to the spacing of mosaic cells, spatial order at the time of cell fate determination, and lateral cell migration.

3.4.2. Does cell fate determination contribute to mosaic regularity?

There are contrasting views as to the role of cell fate mechanisms in determining minimal cell spacing among like cells in the mature vertebrate retina. Newly born chick RGCs have a non-random spatial distribution when still in the apical region of the retina, suggesting that their genesis undergoes spatial constraints (McCabe et al., 1999). Yet, at least in the mammalian retina, most cells forming mosaics migrate to their final layer moving both along (radial) and perpendicular (tangential) to the AB axis, so that their final position is not related to the position in which they were originally born (Reese et al., 1995).

3.4.3. Tangential migration in mosaic assembly

Heterozygous female mice expressing the transgene LacZ linked to the chromosomes X have been used to analyse the spatial relation between retinal neurons and their clone of origin. In these animals, the random X inactivation, which occurs before neurogenesis in the retina, creates a retina from an initial random pattern of LacZ+ (blue) and LacZ- (white) progenitors. If cells in a clone remain together, and only take different AB positions, these clones should be columns arranged along the AB axis. If some cell moves out of the clone, it will stand out as a blue cell in a white column or vice versa. Muller glia, rod bipolar cells and rods, all appear to just migrate radially (i.e. perpendicular to the retinal layers) from the location of their original progenitor. On the contrary, mosaic-forming cells (i.e. cones, horizontal, amacrine, and ganglion cells) are displaced tangentially (i.e. parallel to the retinal layers) away from their clone of origin. The average tangential cell displacement is several tens of microns, and within each mosaic forming population, all the cells move with respect to their clone of origin (Reese and Galli-Resta, 2002; Reese et al., 1995, 1999b). Real-time imaging of cell migration in the zebrafish retina has directly shown tangential cell movements for both horizontal and amacrine cells (Godinho et al., 2007; Poggi et al., 2005b). But which mechanisms drive cell displacement away from like-cells?
3.5. Hypotheses on the nature of homotypic interactions controlling mosaic formation: a role for dendrites?

The finding that in most retinal mosaics neurons avoid being closer to one another than a minimal set distance (reviewed in Cook and Chalupa, 2000; Galli-Resta, 2002), combined with the observation of the almost perfect dendritic tiling that RGC mosaics achieve with minimal dendritic superimposition (Wässle et al., 1983), have suggested the hypothesis that dendrites might be instrumental to cell spacing in retinal mosaics. The following evidence supports this view: (1) Modelling experiments have shown that minimizing dendritic superimposition would suffice to create an orderly cell array (Eglen et al., 2000). (2) In retinas with supernumerary RGCs, the size of the RGC dendritic tree scales in accordance to the increased cell density (Kirby and Chalupa, 1986). Similarly, the dendritic trees of horizontal cells scale with density when different mouse strains are compared (Reese et al., 2005). (3) If all RGCs are killed in a retinal sector, the dendrites of nearby RGCs grow into the lesion, suggesting that interactions between neighbouring RGC dendrites normally control the extent of RGC dendritic trees (Eysel et al., 1985; Perry and Linden, 1982). A similar mechanism, mediated by the cadherin Flamingo, controls dendritic tree development in subsets of Drosophila neurons tiling the fly body (reviewed in Parrish et al., 2007). (4) The cholinergic and amacrine cell mosaics become regular when and where their cells are connected by a continuous dendritic net (Fig. 7). If dendritic stability is inhibited in these mosaics during development, mosaic disorganization occurs (Fig. 8, Galli-Resta et al., 2002).

Dendritic interactions, however, are unlikely to be the only mechanism mediating cell spacing in retinal mosaic, since recent experiments show that a minimal cell spacing is observed in populations of ganglion and amacrine cells that have been so massively depleted in the neonate as to make it unlikely that any dendritic interaction might

Fig. 7. Regular cell spacing in the mosaics of cholinergic amacrine cells and horizontal cells appears when and where a continuous net of dendrites link neighbouring cells: (a) When an incomplete net of processes is observed in the mosaic of cholinergic amacrine cells in the GCL of the rat retina, irregular cell spacing is observed close to the gaps in the net of processes (e.g. cell clusters indicated by arrows). (b) At birth, the cholinergic cells of the GCL are regularly spaced. A continuous net of dendrites links neighbouring elements. (c) On postnatal day 4 (P4), irregular cell spacing and gaps in the net of horizontal cell processes are observed in the peripheral rat retina. (d) At the same age, the horizontal cells are regularly spaced in the central retina, and linked by a continuous net of processes. Confocal images. Scale bar 10 μm. Reproduced with permission from Galli-Resta et al. (2002).
have occurred between like-cells (Farajian et al., 2004; Lin et al., 2004).

3.6. Mosaics as building blocks of retinal assembly?

Independently of the many open questions on mosaic formation, even a very conservative investigator would agree on a few points: (a) most retinal cell types form mosaics; (b) some mosaics form early in development; (c) most mosaics form independently of one another. This allows some speculations. When arrayed in mosaics, like-cells are each warranted an individual limited domain within the retina. If each cell explores a domain centred around its own soma, as many recent experiments suggest that growing dendrites do (reviewed in Lohmann et al., 2002; Wong and Ghosh, 2002), then each neuron can search for synaptic partners only within a limited region. This search will undergo limited competitive interactions between like neurons, each of which will be searching in a region centred on its own domain. Furthermore, if the synaptic partners are also arranged in a mosaic, then topographical order in the connections is ensured, as neighbouring cells in the first array will be connected preferentially to cells that are neighbours in the second array. In this scheme, the capability of cells to recognize one another and two sets of local mechanisms—the minimal spacing rule between like neurons and the local search for synaptic partners—would suffice to lay down a blue-print of retinal architecture (Galli-Resta, 2001, 2002).

3.7. Exploring the link between mosaics and layer formation

Speaking of mosaics as the two-dimensional arrays that can be seen in retinal flat mounts, one tends to forget the basic question of how mosaic cells become confined within a single cell monolayer. This question is still unanswered, but there are interesting indications. The horizontal cells are initially moving across the retinal thickness (Edqvist and Hallbock, 2004), and later converge to a single retinal layer, acquiring extra-regularity as they do so (Novelli et al., 2007; Raven et al., 2005). Similarly, the early cholinergic amacrine cell mosaics occasionally display cells out of the mosaic layers, before acquiring their adult-like regularity (Galli-Resta et al., 2002, 1997). Furthermore, if the dendrites of these cells are temporarily perturbed (Fig. 8), this disrupts both the regular spacing of the cells and their alignment in a single cell monolayer (Galli-Resta et al., 2002). These data indicate a strong correlation between mosaic and layer formation, but before discussing this point further, we need to consider what is currently known about layer formation.

3.8. Mosaic development in short form

Mosaics are orderly arrays of like-cells that occupy a single cell monolayer. Commonly, their geometrical regularity derives from the presence of a minimal spacing between their cells. The first amacrine cells and cone mosaics appear before their layers can be told apart (Fig. 6). The formation of mosaics seems predominantly
due to interactions that are restricted to the mosaic cells, as they appear indifferent to perturbations affecting other cell types. Several processes collaborate in controlling mosaic formation, including cell death, and lateral tangential migration. A number of studies show a strong relationship between dendritic development and cell spacing in retinal mosaics. Some mosaics become regular where and when their cells are linked by a continuous net of dendrites (Fig. 7), and perturbing this net disrupts regular cell spacing within the mosaic, as well as its alignment in a monolayer (Fig. 8). These observations suggest a link between mosaic and layer formation.

4. Investigating the mechanisms controlling cell layering

The arrangement of cells in layers is the first feature of the retina described in textbooks. From the point of view of building strategies that each cell type has a layer to migrate to, as a part of its differentiation program seems very efficient. If we consider the building of the retina as an incessant sorting process taking place while many events occur simultaneously (cell migration, cell divisions, cell birth, cell death, differentiation, process outgrowth, etc.), a strategy of “bucket sorting” seems advantageous. As it is generated, each element goes to its “bucket”/layer, where it is spatially close to the elements to which it will eventually connect. In many severe retinal pathologies, retinal layering is disrupted or altered, which is the most obvious indication of the importance of this organization. But how are layers built in the making of the retina?

During the last few years, a considerable number of studies have shown the effect of genetic or other experimental manipulations on the retinal organization in layers, or, as some refer to it, its lamination. As we will see, an emerging aspect is that neurogenesis and lamination can be viewed as separate processes, regulated by different control mechanisms. For this reason, here we will purposefully ignore experiments affecting the generation of specific cell types, although they have obvious consequences on lamination without apparent effects on the genesis of the different retinal neurons. Neurogenesis and cell fate control in the retina have been the subject of recent reviews to which the reader is referred (Cayouette et al., 2006; Livesey and Cepko, 2001; Rapaport, 2006).

Very schematically, the manipulations leading to alterations affecting the normal development of retinal layers identified so far can be subdivided in three major categories: (a) alterations targeting classic adhesion molecules or the basal lamina, (b) alterations affecting the proteins of the Crumbs (crb)- or the Apical Polarity complex, and (c) alterations removing diffusible signalling factors. Following is a detailed illustration of these effects. The reader wishing to skip this presentation can directly go to the summary at the end of this Section 4.4.

4.1. Role of classical adhesion molecules and basal lamina

4.1.1. Basal lamina

Since the stage of proliferating neuroepithelium, the retina is delimited on its vitread side by a basal lamina, the ILM, a “fabric” made by laminin and collagen fibres and other ECM molecules. A number of studies illustrate alterations in the inner retinal architecture due to mutations or manipulations affecting the ILM. Several hereditary diseases in humans, such as Fukayama muscular dystrophy, muscular-eye-brain diseases and Walker–Warburg syndrome show breaches in the ILM and associated retinal ectopias—i.e. clumps of neurons exiting the inner retina boundary toward the vitreous (Hallia et al., 1997; Nakano et al., 1996; Sertie et al., 2000; Williams et al., 1984). Random ruptures of the ILM and associated retinal ectopias (Fig. 9) are also observed in mice KO for components of the ILM (e.g. nidogen (Candiello et al., 2007), or laminin γ-1 (Halfter et al., 2005)). These mice often display extensive retinal haemorrhages due to retinal vascular leakage, but ectopias seem likely to be due to ILM ruptures, as the two are spatially associated. The causal link between inner retinal disorganization and ILM loss is further supported by experiments in chick, where the lack of retinal vasculature allows using enzymatic digestion to target the ILM without vascular complications. Intraocular injection of collagenase during early retinal neurogenesis in chick embryos completely digest the ILM in 24 h and leads to an altered stratification of RGC somata within a few days (Halfter and Schurer, 1998).

4.1.2. Receptors for extracellular matrix components: integrins and dystroglycan

Integrins are the major family of cell adhesive receptors binding components of the ECM. Their binding to ligand leads to the formation of focal adhesions that link the ECM to the intracellular cytoskeleton (reviewed in Hynes, 2002).

In the developing retina, integrins are found throughout the retinal layers (Cann et al., 1996). Mouse embryos lacking integrin z6 (Georges-Labouesse et al., 1998) or both z3 and z6 (De Arcangelis et al., 1999) display retinal abnormalities, with ectopic neuroblastic outgrowths in the vitreous body in the eye and altered ILM deposition. In the embryonic chick retina blocking integrin β1 prior to neurogenesis dramatically reduces retinal growth and disrupts retinal neuroepithelial integrity (Svennevik and Linser, 1993). Similarly, blocking integrin β1 in Xenopus embryos causes anomalous retinal lamination, with abnormal cell clusters in the outer retina, commonly known as rosettes (Li and Sakaguchi, 2004). Rosettes (Fig. 10) are sphere-like structures where the relative stratification of neurons is locally correct, but it is organized inside-out, with the apical segments of the photoreceptors pointing inward.

In addition to integrins, another transmembrane receptor linking the ECM to the cytoskeleton is dystroglycan
DG is expressed in the developing retina, and is specifically localized to the neuroepithelial endfeet at the vitreal border (Blank et al., 1997). Antagonizing DG function in the embryonic chick retina disrupts the AB orientation of neuroepithelial cells, and causes hyperproliferation, while opposing effects are obtained by overexpressing DG (Schroder et al., 2007). In Xenopus, loss of DG function leads to ocular malformations including microphtalmia, early disorganization of the basal lamina, and clustering of rosette-like structures (Fig. 10) in the mature animal (Lunardi et al., 2006).

4.1.3. Cadherins and their intracellular ligands

Cadherins form a superfamily of transmembrane molecules, many members of which appear to act as Ca\(^{2+}\)-dependent adhesion molecules (reviewed in Takeichi, 1995). Several cadherins are expressed in the vertebrate eye, and some (N-cadherin, R-cadherin, cadherin-11, cadherin-8, and cadherin-6) dynamically change their expression during retinal development (Honjo et al., 2000).

N-cadherin is a major component of the apical belt of AJs joining the neuroepithelial cells of the developing retina. In the presence of functional blocking antibodies against N-cadherin, explanted chick retinas of early embryos tend to dissociate and cannot be maintained as a tissue mass (Fig. 11a and b). Older retina do not disaggregate but develop abnormal arrangements of cells in the retina, leading to the appearance of rosette-like cell clusters (Matsunaga et al., 1988). Similarly, severe alterations of retinal architecture have been observed in different Zebrafish mutations affecting the coding region of the N-cadherin gene (Erdmann et al., 2003; Malicki et al., 2003; Masai et al., 2003; Pujic and Malicki, 2001). In these mutants, the AB polarity of the proliferating retinal neuroepithelium is dramatically compromised (Fig. 11d and e) in the embryo, and rosette-like patterns form in the mature retina (Fig. 11f and g).

Fig. 9. RGC ectopias associate to ILM breaches: (a, b) Sections through the eye of a control (a) and a laminin-γ1 KO mouse (b) at 16.5 days of gestation. Note the haemorrhages in (b-d). Radial sections of the normal (c) and laminin-γ1 KO retina (d). The control retina has a smooth vitreal (basal) surface (black arrow) with multiple hyaloid arteries (white arrows), whereas cellular ectopias (E) are seen along the vitreal surface of the mutant retina (d–f). Electron microscopic images of the basal surface of the retina of the normal (e) and laminin-γ1 KO retina (f). A continuous, uninterrupted ILM (arrow) is seen in the control retina (e), while mutant mice have a discontinuous ILM (f). R = retina, L = lens, ON = optic nerve. Bar: (a, b) 100 μm; (c, d) 50 μm; (e, f) 50 nm. Reproduced with permission from Halfter et al. (2005).
Knock down of R-cadherin in Zebrafish leads to a spectrum of effects, where the most severely affected animals have small eyes, increased cell death and lack of retinal lamination (Babb et al., 2005). Silencing the Xcadherin-6 gene in Xenopus, leads to abnormal eyes showing lamination defects with rosettes on the outer retina, a reduced GCL and a fragmented RPE (Ruan et al., 2006).

In the AJs, cadherin molecules are linked to the actin cytoskeletal network. An essential component of this link is β-catenin, which is abundantly expressed in the developing retina (Liu et al., 2002; Mu et al., 2001). Conditional KO mice that silence β-catenin expression in the retina just before neurogenesis display a normal proliferation rate, but retinal progenitor cells loose their normal AB arrangement. During subsequent development, these retinas exhibit a higher than normal rate of cell death and display rosette-like cell aggregates (Fu et al., 2006).

4.2. The crb and Apical Polarity complexes

A highly conserved molecular machinery positions AJs at the interface between apical and basolateral plasma membrane domains in epithelia. Major components of such machinery are the crb and Apical protein complexes (reviewed in Richard et al., 2006; Tepass and Harris, 2007). Not surprisingly, mutations affecting either complex affect the proper development of retinal layers.

In Drosophila, crb is necessary for the cohesion of the epithelia, and for proper eye development (Tepass et al., 1990). Mouse mutants in crb1 (one of the two crb homologues), show a fragmented outer limiting membrane (OLM, the mature form of the AJs belts), photoreceptor degeneration, rosettes formation and retinal folding (Mehalow et al., 2003; van de Pavert et al., 2007). In humans, mutations in the crb1 gene are observed in 4% of patients affected by retinitis pigmentosa and in 10–15% of Leber congenital amaurosis (Richard et al., 2006). Interestingly, analysis of the retina in a patient carrying a crb1 mutation showed abnormal layering (Jacobson et al., 2003). Mutations or pharmacological manipulations affecting elements of the crb complex in Zebrafish cause disruption of the AB polarity in the retinal neuroepithelium, and severe alteration of retinal lamination, rosette formation and abnormal photoreceptors in the mature animal (Hsu et al., 2006; Malicki and Driever, 1999; Omori and Malicki, 2006; Wei and Malicki, 2002; Wei et al., 2006).

The Apical Polarity complex (comprising Par3, Par6 and atypical PCK), appears to physically interact with the crb complex in vertebrates. All known mouse and zebrafish mutations affecting proteins of the Apical Polarity complex, display retinal delamination and rosettes (Koike et al., 2005; Malicki et al., 1996; Richard et al., 2006; Wei et al., 2004).

4.3. Diffusible factors and the role of RPE: retinal re-aggregates and transplants.

The mechanisms controlling the development of retinal architecture have also been investigated by disaggregating embryonic retinal cells and allowing their re-aggregation and development in tri-dimensional cultures. Retinal cells re-aggregate in spheroids, where mitotic divisions are localized in rosette-like patterns. These give rise to all different retinal cell types that locally stratify according to the normal order observed in the retina. The differentiated re-aggregates however do not present the continuous laminar retina-like organization, because often photoreceptors are found pointing inside-out, in rosette-like patterns (Fig. 12a and b). A continuous correct lamination is observed when the re-aggregates are allowed to grow in media conditioned by Muller glia or RPE cells (reviewed in Layer et al., 2002), or (Fig. 12c and d) when cultured together with explants of the embryonic retinal anterior rim (Nakagawa et al., 2003). These experiments indicate
the need of diffusible factors to orchestrate global retinal organization. The nature of these factors is still unknown (but see Nakagawa et al., 2003). The importance of the RPE in controlling the development of retinal architecture has been confirmed in vivo, by selective genetic ablation of the RPE cells. Loss of RPE during retinogenesis is associated to microphthalmia, disorganization of the retinal architecture, and the appearance of rosettes. Interestingly, where some patches of RPE escaped ablation, the correct laminated structure of the retina was maintained adjacent to the RPE cells (Raymond and Jackson, 1995).

Interestingly, rosette-like patterns are also commonly observed when embryonic retinas are transplanted ectopically in the brain. These transplants produce all the normal retinal cell types, are usually smaller than normal retinas and connect to the retino-recipient visual centres of the host brain (reviewed in Lund et al., 1988). Behavioural and electrophysiological studies show that transplanted retinas transmit light information to the host brain (Coffey et al., 1989; Klassen and Lund, 1987) albeit lacking topographical mapping in their connections (Galli et al., 1988). This agrees with the view suggested by their anatomical organization, i.e. that the circuitry is locally correctly wired, but it is not organized in a global map.

4.4. A summary of layer development: common end points for common mechanisms?

Reviewing the effects of mutations and manipulations affecting layer development, some common themes emerge. When the basal side of the retina is affected (experiments on the ILM and some integrins), the main effects are on the inner retina, with local cell delamination and ectopias, as if the retina had lost its inner (basal) border (Figs. 9 and 14). Note however, that some experiments affecting integrins and DG have more severe effects, interesting the entire retina, as does the expression pattern of these proteins.

When the retinal apical side is affected, and in particular, the AJ's, dramatic effects may ensue, from total retinal de-aggregation (loss of N-cadherin in early retinas; Fig. 11a and b), to the lack of any retinal lamination (R-cadherin mutants), or the loss of neuroepithelium AB polarity, and subsequent retinal delamination (Fig. 11c–f; N-cadherin Zebrafish mutants, Zebrafish mutants in crb-complex components, mouse and Zebrafish mutants in Apical Polarity complex components, β-catenin conditional KO mouse, RPE ablation). Not all the mutations affecting the apical side are so severe. Some display their effects mostly in the phase of photoreceptor maturation (e.g. crb1 mice and human mutants), but this might depend on the partial redundancy of the molecules involved (Richard et al., 2006).

By far, the most common abnormality in retinal laminations is the appearance of rosette-like cell clusters, which can either affect only cells of the outer (apical) retina or all retinal layers. Rosettes, which are commonly observed in a number of retinal pathologies, are characterized by the presence of a locally correct cell stratification, which is not continuous throughout the tissue, as photoreceptors point inside-out (Figs. 10 and 12). In retinal re-aggregates this defect is prevented by soluble factors secreted by the RPE (Fig. 12b). Conversely, retinas lacking the RPE show rosettes.
A very schematic hypothesis to order these effects could be as follows: AB polarity in the neuroepithelium seems essential for the development of the appropriate continuous retinal stratification in layers. Normally, the RPE acts as a “polar star”, producing factors that allow positioning and alignment of the apical sides of the neuropithelial cells. The main feature of the apical side appears to be the presence of the AJs. Manipulations affecting AJ components (N-cadherin, β-catenin, etc.), or the complexes controlling their formation (crb and Apical complexes), compromise layer formation. Finally, a normal retinal layering also requires that an appropriate basal lamina is deposited at the basal side, and that cells have the right type and amount of integrins to connect to it.

Independently of the validity of this schematic view, there is a clear predominance of adhesion molecules and their binding partners among the molecules so far known to affect retinal patterning. Thus, it seems reasonable that, alongside biochemical and genetic investigations, one important avenue to explore is the role of adhesion and, more generally, of mechanical interactions in shaping the retinal tissue. This exploration is just beginning. The following section outlines some of its important and unexpected aspects.

5. An emerging role for mechanical forces in retinal development?

Retinal pathologists and ophthalmic surgeons experience the impact of mechanical forces on the retina on a daily basis, from retinal detachment, to excavation of the optic nerve head in glaucoma, or retinal tears caused by vitreo-retinal traction, to mention a few. Yet, mechanical forces are largely neglected in the study of normal retina development.
5.1. Mechanical components in the control of eye size

A role for mechanical forces in eye development has been suggested by classical embryology. In the 1950s, Coulombre showed that intraocular pressure (IOP) promotes eye growth. Inserting a hollow cannula into the eye, which allowed vitreous efflux and thus reduced IOP, he was able to almost abolish the 6-fold increase observed in the eye of the chick embryo between embryonic day 4 (E4) and E8 (Fig. 13a). The same effect was not obtained using a solid rod of the same size as the cannula (Coulombre, 1956). The force exerted by IOP is counterbalanced by the sclera (reviewed in McBrien and Gentle, 2003), and, at least during embryonic development, also by the ILM. Enzymatic digestion of the ILM with collagenase injection in the E4 chick embryo causes a 50% increase in eye size within 4 days (Fig. 13b), an increase which is almost completely prevented by promoting ILM reconstitution with a chasing injection of laminin (Halfter et al., 2006). These experiments, and the growing field of studies on the mechanisms controlling eye growth in animal models of myopia, indicate an important role for mechanical forces in global eye growth. But what about the retinal tissue and its organization during development?

5.2. Naïve considerations on retinal cohesion during development

To explore whether mechanical forces have a role in retinal development, we will start with some naive considerations. Electron microscopy studies show that the retinal neuroepithelial cells are anchored basally to an ECM lamina (the ILM), while apically, they are joined to one another by belts of AJs. These connections are maintained throughout the cell cycle, so that even during mitosis dividing cells remain in contact with adjacent cells, and clones generated by single precursors radiate as compact distributions of cells from the centre to the periphery of the retina (Reese et al., 1995). Can we envision cell adhesion to the ILM and cell–cell adhesion through AJs as the main forces that hold the retinal neuroepithelium together while it grows?

The most striking evidence in support of this view is the observation that early functional removal of N-cadherin, the core protein of AJs, leads to a total de-aggregation of the retina (Fig. 11a and b Matsunaga et al., 1988). Subsequently, this or other manipulations affecting N-cadherin, are not so effective (Fig. 11c–f), as AJs are not lost, but rather become spatially disorganized. This disorganization suffices to prevent the global orderly development of layers and mosaics, and commonly rosette-like patterns form (Erdmann et al., 2003; Malicki et al., 2003; Masai et al., 2003; Matsunaga et al., 1988; Pujic and Malicki, 2001). Intuitively, this is not surprising. If adhesion between cells is not homogeneous and/or not adequate to balance the forces that expand the tissues, one should expect tissue disruption at one extreme, and in general, tissue irregularities. We could even naively envision that, if AJs are stronger on one side of a cell than on the opposite side, the cell might be pulled sideways, and out of the AB orientation. Once the AB orientation of the tissue is locally perturbed, this could easily evolve into folds and rosette-like patterns as cells proliferate. This is of course a speculation, but it serves to consider how mechanical unbalance should be expected to generate tissue irregularities.

There are also striking indications for a mechanical action of the ILM in maintaining tissue integrity. Mice or men with defective ILM and/or integrins (the receptors that cells use to anchor the ILM) show a similar phenotype (Candiello et al., 2007; De Arcangelis et al., 1999; Georges-Labouesse et al., 1998; Halfter et al., 2001; Haltia et al., 1997; Nakano et al., 1996; Sertie et al., 2000; Williams et al., 1984). In the absence of the ILM, the cells of the
inner retina are disorganized (Fig. 14a and b), while in the case of a discontinuous ILM, they bulge out of the ILM breaches into the vitreous body (Fig. 9). These behaviours strongly suggest that normally the ILM mechanically constraints the retinal neuroepithelium. Interestingly, hours after ILM digestion in the chick embryo eye, the neuroepithelial cells retract their basal processes (Fig. 14c and d), as if, having lost their attachment to the ILM, they had lost tension and recoiled (Halfter, 1998). To understand whether these are just metaphors or real events, one needs to explore the mechanical properties of the developing retina.

5.3. The mechanical properties of retinal tissue and cells

Atomic force microscopy probes the mechanical properties of tissues on very small spatial scales, using cantilevers with nano- to micro-metric tips that, “tapping” on the sample, allow the determination of its deformation in response to an applied force. When so tested, the chick ILM displayed a predominantly elastic behaviour, with an elastic modulus (a measure of the “spring like” properties of the material) similar to the values obtained for cartilage tissues (around 5–10 kPa Candielo et al., 2007). This suggests that the ILM is a flexible membrane.

Fig. 14. ILM degradation disorganizes the GCL and causes the retraction of the basal endfeet of neuroepithelial cells: (a, b). Sections from a normal 5-day (E5) chick embryo retina (a) and an E5 retina 24h after digestion of the ILM with collagenase. The ILM is immunolabelled in red (a). Note the disorganization of the GCL. (c, d). Single neuroepithelial cells (green) in a normal (c) and collagenase treated E5 chick retina. The basal endfeet of the neuroepithelial cells reach the ILM (red) in normal retinas (c), but withdraw from the basal surface when the ILM is degraded (d). The arrows indicate the retinal apical margin. Reproduced with permission from Halfter et al. (2006) (a, b), Halfter et al. (2001) (c, d).
that can transmit forces across the tissue, and resist cell traction, acting as an external scaffold, provided that neurons are softer than the ILM. Is this the case?

Testing the mechanical properties of acutely isolated retinal cells by scanning force microscopy, has shown that both retinal neurons and Muller glia have a dominant elastic component (i.e. they deform in proportion to the applied stress and tend to recover their initial shape once stress is over), and are very soft (elastic modulus 200–600 Pa), indeed among the softest tissue of the body. Similar properties are observed when macroscopically testing retinal explants (Lu et al., 2006). Unfortunately, there are no direct measurements of the mechanical properties of individual retinal cells during development, but they are likely to be as soft or softer than adult neurons since whole retinal measures showed a stiffening of the retina with age (Reichenbach et al., 1991). In support of this, culture studies show that typical neuronal elastic modules are around hundreds of PA, suggesting that this may be a general feature of neurons (Georges and Janmey, 2005; Georges et al., 2006). Putting these data together we can look at the cells forming the retinal neuroepithelium as soft springs anchored on their basal side to a much stiffer continuous scaffold, the ILM. Furthermore, considering the total retinal de-aggregation induced by early N-cadherin perturbation, adhesion at the levels of the AJs seems even more important than the ILM in holding the tissue together, at least in the young retina, when the ILM is thinner and weaker than at later stages (Candiello et al., 2007). However, the mechanical role of AJs in keeping the vertebrate retina together during development still needs to be explored in more details.

The view that AJs provide direct physical coupling between adjacent cells and endow cells with the capacity to act as a syncytium is a common developmental theme (see for example Gumbiner, 1996; Halbleib and Nelson, 2006). Compelling data come from the Drosophila, where the dynamic regulation of AJs is an essential feature of epithelial cohesion and growth. Recent studies show that epithelial cell patterns might minimize mechanical interactions between the cells (reviewed in Lecuit and Lenne, 2007). Remarkable examples are the clusters of four lens cells found in each ommatidium of the Drosophila eye. At maturity, the cluster of lens cells is strikingly similar to a cluster of soap bubbles, which assumes its configuration by minimizing surface tension (Fig. 15). This similarity is also observed in mutants presenting clusters of 3–6 lens cells. Genetic manipulations and modelling studies suggest that patterning of these cell clusters depends on a force equilibrium in which adhesion mediated by E- and N-cadherin opposes the inner cell tension generated by the action-myosin cytoskeleton (Hayashi and Carthew, 2004; Janmey and Discher, 2004; Kafer et al., 2007). It will be interesting to explore whether similar mechanisms control the lateral migration and compaction of the cone photoreceptors forming the primate fovea (reviewed in Curcio et al., 1990), where the cone mosaics assumes an almost crystalline geometry.

5.4. The beginning of retinal layers: a mechanical explanation for RGCs basal migration

Thus far, we have been dealing with dynamic mechanical forces that hold the retina together while it grows. In a way, the appearance of layers and mosaics occurs as cells make their controlled escape from this strong AB cohesive mechanism, since neuronal differentiation in the retina begins with cells losing contact with either one (e.g. the RGCs) or both (e.g. amacrine cells) of the AB edges.

Is a mechanical view also interesting to investigate these processes? On the basis of their real-time observations of RGC differentiation, Zolessi and co-authors suggest that, once the axon is formed, the RGC body moves to the basal surface because that is where the cell remains attached when the apical side retracts (Zolessi et al., 2006). Similarly, a century ago Cajal hypothesized that the growth cone pulls the cell body towards the basal surface to which it is attached (Ramon y Cajal, 1929). In agreement with this view, ILM degradation leads to RGCs scattering away from the ILM (Fig. 14a and b), and disorganizes the course of their axons (Haffter, 1998; Haffter et al., 2005). Furthermore, studies in cultures of

Fig. 15. A balance between cell adhesion and inner cellular tension appears to control the patterning of lens cells in the Drosophila eye: (a) The Drosophila eye, formed by multiple ommatidia. (b, c) The clusters of four lens cells normally found within each ommatidium (b) closely resemble clusters of soap bubbles (c). Adapted from Janmey and Discher (2004) by permission from Macmillan Publishers Ltd.: Nature, copyright 2004.
different neuronal types support this view. In an elegant series of studies, Heidemann and collaborators have investigated the role of mechanical tension in regulating axonal growth, using calibrated needles to produce or resist measurable forces (reviewed in Heidemann et al., 1995). These studies have shown that, as the growth cone advances, it pulls on the trailing neurite (Heidemann, 1996; Heidemann and Buxbaum, 1991; Heidemann et al., 1990; Zheng et al., 1994), and this tension can drag the soma, like a dog on a leash (Lamoureux et al., 1989). In accordance with this view, studies in zebrafish mutants show that in cases of altered AB polarity, RGC axons reach the closest ECM basal lamina, either the ILM or the Bruch membrane behind the RPE, and the cell bodies accordingly follow (Zolessi et al., 2006).

### 5.5. Cell sorting and the differential adhesion hypothesis

While RGCs may be dragged towards the basal lamina by their growth cones, nascent amacrine cells seem to behave rather differently. They migrate without retaining their anchoring to the apical and basal side, (although they might move along AB-oriented neuroepithelial cells), and stop when they get close to the RGCs (Godinho et al., 2005). How do they know where to stop? It could be conceived that molecular markers exist that distinguish different depths along the AB axis. In the mature retina, the search for markers that could differentiate sub-laminae within the IPL, has shown that the Sidekicks adhesion molecules and several known cadherins have a laminated or regionalized pattern of expression (Yamagata et al., 2002). A regional code based on differential expression patterns of adhesion molecules might get even more diversified when considering the protocadherins (Takeichi, 2007). These differential expression patterns have been observed only in the mature retina, but are undoubtedly suggestive of a potential role of differential adhesion (DA) in positioning different cells at different depth during retinal development.

Cell sorting based on DA has been hypothesized following classic embryological experiments where cells disaggregated from two different tissues (e.g. liver and retina) initially start as a mixture and gradually become sorted in a tri-dimensional aggregate where one of the cell type usually envelops the aggregate formed by the cells of the second type (Fig. 16, Steinberg, 1996). The “DA hypothesis (DAH)”, explains this behaviour by comparing cells to liquid molecules, and suggests that tissues formed by cells endowed with different surface adhesion properties sort out like immiscible fluids (reviewed in Steinberg, 2007). In agreement with this view, culture experiments show that cells of different types always segregate according to the relative surface tension of their aggregates (Fig. 16). Moreover, when cell lines are used that only express cadherins, the surface tension of their aggregates is directly proportional to the number of cadherin molecules expressed on the cell, thus linking the global property that dictates the sorting behaviour to the individual adhesive characteristics of the single cells (Foty and Steinberg, 2005). There is still controversy about the DAH, and it is possible that a model assuming a liquid-like cell sorting behaviour will need the complement of the cell elastic components. Yet, at present, this hypothesis is rather appealing, and it will be interesting to understand whether and how much it may explain differential cell positioning in the developing retina.

### 5.6. Tension induces process outgrowth and stabilization

The hypothesis that axons elongate in response to the mechanical tension exerted by the growth cone is more than a century old, but its first experimental evidence is much more recent. In 1984, Bray showed that axons of cultured cells can be elongated by experimentally applied tension (Bray, 1984). Then, Heidemann and collaborators performed a systematic quantitative analysis of this problem in cultured neurons (Chada et al., 1997; Heidemann, 1996; Heidemann and Buxbaum, 1990, 1994; Heidemann et al., 1995, 1999; Joshi et al., 1985; Lamoureux et al., 1992; Zheng et al., 1991, 1993). They found that there are two major regimes of tension effects on neurons, a non-growth, elastic regime, and a fluid-like growth regime.
The elastic behaviour (i.e. a deformation from which the cell recovers as soon as tension is lost) is observed below a certain threshold. When tension goes beyond threshold, it initiates process outgrowth if applied on the cell body, or stimulates outgrowth if exerted on existing processes (Fig. 17). In all cases, a surprisingly robust linear relationship is observed between the process elongation rate and the tension applied. These studies have also shown that neurites are constantly producing an internal tension, and that this underlies their retraction when anchoring is lost (reviewed in Heidemann, 1996; Heidemann et al., 1995). Interestingly, the strength of cadherin mediated adhesion, as determined by single bond rupture measurements, has an average force per bond around 50 pN (Leckband and Prakasam, 2006), which is about $\frac{1}{10}$ of the force necessary to initiate or stimulate neurite outgrowth in culture (10–50 µdyn, reviewed in Heidemann et al., 1995). Thus, it is conceivable that as soon as the developing dendrites of retinal cells are engaged by a membrane presenting a few cadherin molecules on its surface, this may suffice to stabilize them and promote their elongation. In line with this view, when N-cadherin is selectively ablated in horizontal cells, their dendrites shrink (Tanabe et al., 2006), as do the dendrites of RGCs when integrin-mediated cell adhesion is selectively hampered in these cells (Marrs et al., 2006).

5.7. The cell ‘‘tactile set point’’

The growth promoting effect of tension cannot explain the selective stabilization of the cell processes in the plexiform layers that is observed in vivo (Godinho et al., 2005; Morgan et al., 2006), unless we go back to the hypothesis of an adhesion-molecule-code for selective locations (Yamagata et al., 2002). This hypothesis is undoubtedly fascinating, but admittedly, a subdivision of the IPL in terms of adhesion molecule expression is evident in the mature retina (Yamagata et al., 2002), and not before the IPL forms. Yet, amacrine cell processes selectively stabilize in a region that thus becomes the IPL (Godinho et al., 2005). There is still no explanation for this behaviour, but mechanical studies of neuronal growth in response to matrix stiffness provide an attractive hypothesis. Recent findings show that cell behaviour depends on the stiffness of the substrate on which the cell grows. This includes whether the cell lives, dies, proliferates and whether and how it differentiates. In particular, each cell type seems to have a preferential interval of substrate stiffness that optimizes cell differentiation. This “tactile set point” is close to the stiffness of the tissue the cell belongs to in the organism (reviewed in Discher et al., 2005). Neurons prefer soft substrates, and when all other parameters are alike, the stiffness of the substrate determines whether neurons or astrocytes survive in primary cultures obtained by dissociating different brain regions. When neurons find their optimal, soft substrate they develop numerous processes (Georges et al., 2006).
Thus, the selective stabilization of processes in the region of the future IPL could be explained if this region is softer than the adjacent GCL and NBL regions, mostly occupied by cell bodies. Interestingly, the measured mechanical properties of retinal neurons suggest that this could be the case, as there are sub-cellular variations in elasticity, with the cell processes being softer than the cell somata, possibly for the rigidity of the nucleus (Lu et al., 2006). It will be interesting to directly test this hypothesis in the future.

5.8. The strain-stiffening behaviour of cells and biological polymers

Why is it that we talk of elastic behaviour in one case, proposing that the RGC cell body is dragged as a whole by the growth cone, while in a different instance we describe the fluid-like behaviour of processes stimulated by applied tension? In other words, why can we envision cells responding to pulling forces with global movement in one instance, and with just a local process protrusion in a different context?

The answer to this question lies in the strain-stiffening behaviour of cells, which is the property by which cells become stiffer as they are deformed. Strain-stiffening characterizes cells as well as biological polymers, such as actin and neurofilaments gels (reviewed in Heidemann and Wirtz, 2004; Janmey and McCulloch, 2007; Janmey and Weitz, 2004). Indeed, strain hardening in cells seems to be mostly due to this same property displayed by their cytoskeleton. Schematically, and very improperly, this property derives from the fact that the filaments in cytoskeletal networks are not straight, and have many small bends. When pulled, they initially just straighten out, with little resistance. Once they are stretched out completely, however, additional pulling will encounter much more resistance, as it now acts against the intrinsic elasticity of the filaments. This behaviour can be easily observed using a folded piece of paper: it is much easier to pull out the folds than to stretch the paper itself.

Strain hardening is a rather advantageous property, as it protects cells and tissues from tearing. It also allows the cell to avoid major changes in response to small local forces, and to respond globally to a larger force. Thus, responding to small forces by elongating a local process and to stronger forces by moving as a whole, appear to be both in the repertoire of neuronal reaction to forces.

5.9. A mechanical link between mosaics and layers?

To conclude this panorama of mechanical themes, we go back to the idea of potential links between mosaic and layer formation. Immunohistochemical observation and quantitative analysis show mosaics that become regularly spaced where and when a net of dendrites links neighbouring cells (Fig. 7), suggesting that dendrites might control cell positioning (Galli-Resta et al., 2002). In light of our present knowledge of neuronal responses to mechanical forces, we can envision a neuronal net formed by like cells, where mechanical forces act on neurites and somata, dragging, pushing, and pulling, until an equilibrium is achieved that minimizes the forces. If we assume that the cells forming a mosaic are mechanically homogeneous, which seems reasonable as they are all of the same type, then their equilibrium configuration will be a monolayer (to minimize forces perpendicular to the net) of roughly equally spaced cells (to minimize forces parallel to the net). This mechanical model also explains why manipulations affecting the cell dendrites simultaneously disrupt both the orderly cell spacing and their mono-layered arrangement (Fig. 8) (Galli-Resta, 2002; Galli-Resta et al., 2002). It also provides a simple explanation for the observation that retinal cells forming mosaics move laterally with respect to their clone of origin at the same time as they become aligned in a single monolayer (Reese et al., 1999a). Whether and for which cells a mechanical explanation accounts for regular cell spacing within a monolayer will need to be further explored.

6. Future directions

This review is not meant to under-rate the complexity of the biochemical and genetic mechanisms controlling retinal development, but simply to suggest that mechanical stimuli may be as important as chemical stimuli in tissue development.

Several major “mechanical” questions are still unanswered, in the retina as elsewhere. These include the nature of the cell mechano-sensors, the extraordinary mechanism by which mechanical tension can integrate the complex biochemistry of process elongation, and many others. Yet, although still mainly speculative and incomplete, a mechanical view seems very promising, not only to investigate retinal development, but also for its potential application in retinal pathologies. There are too many venues that can be explored from this viewpoint to mention them all, so we end with just a few examples.

In the field of retinal prosthesis and transplants, knowledge of the effects of matrix stiffness on cell outgrowth and differentiation (reviewed in Discher et al., 2005) may help devise specific substrates that selectively promote neuronal rather than glial growth. Interestingly, recent experiments show that transplanted stem cells integrate much better in retinas lacking the mechanically “stiff” astrocytes, than in normal retinas (Kinouchi et al., 2003), an observation in line with the importance of soft substrates to promote neuronal growth and differentiation.

Another interesting area to explore is the role of cell adhesion in synaptic remodelling and in the atrophy and/or abnormal outgrowth of dendrites observed in retinal pathologies such as retinitis pigmentosa (Marc et al., 2003), or in normal aging retinas (Liets et al., 2006).

A third area of considerable interest derives from the growing evidence that master genes, such as Pax6, might
exert some of their most dramatic effects on eye development by controlling cell adhesion properties, thus regulating for example, the interface between the optic vesicle and the lens placode during induction (reviewed in Collinson et al., 2004).

Biomechanical investigations are experiencing a “renaissance”, thanks to the ever-improving technical sophistication of the available experimental tools, and to the growing collaborations between biologists, physicists and engineers. Future break-throughs are soon to be expected.

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