OPINION

Intrinsically different retinal progenitor cells produce specific types of progeny

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Abstract | Lineage studies conducted in the retina more than 25 years ago demonstrated the multipotency of retinal progenitor cells (RPCs). The number and types of cells produced by individual RPCs, even from a single time point in development, were found to be highly variable. This raised the question of whether this variability was due to intrinsic differences among RPCs or to extrinsic and/or stochastic effects on equivalent RPCs or their progeny. Newer lineage studies that have made use of molecular markers of RPCs, retrovirus-mediated lineage analyses of specific RPCs and live imaging have begun to provide answers to this question. RPCs that produce two postmitotic daughter cells — that is, terminally dividing RPCs — have been the most well characterized RPCs to date, and have been shown to produce specific types of daughter cells. In addition, recent studies have begun to shed light on the mechanisms that drive the temporal order in which retinal cells are born.

The vertebrate retina has a highly organized and conserved structure[1] (FIG. 1a). It acts as a miniaturized parallel processor with channels dedicated to the extraction of useful features from the visual scene[2]. The transformations of photoreceptor signals into these features are carried out by a repertoire of more than 60 retinal cell types[3]. The fascinating question of how this cellular diversity is generated during development has been the focus of many studies.

All cells in the retina are derived from retinal progenitor cells (RPCs). There is an evolutionarily conserved order of genesis of the neurons and glia of the retina[4–8] (FIG. 1b) that may reflect the order in which these cell types evolved (BOX 1). The classic birth-dating studies that demonstrated the approximate order of genesis of the different classes of cells have recently been augmented by studies that show that specific subtypes of retinal cells are also born in a particular order[9–11] (FIG. 1c). These observations suggest that temporally regulated mechanisms are a major factor that drives cell fate outcomes.

Early lineage studies in several species[12–17] showed that RPCs are generally multipotent. The clones (BOX 2) produced by RPCs dividing at particular stages of retinal development were labelled using multiple methods, including infection by retroviruses, injection of intracellular tracers, live imaging or use of genetic markers. Retroviral infections of the embryonic mouse retina generated a great diversity in the size of clones (ranging from 1 to 234 cells) as well as highly variable cellular composition[17]. Similarly, clone composition was highly variable, even in the smaller clones derived from RPCs infected in the later, postnatal period[13,17]. Even experiments tracing the outcome of what are presumed to be the terminal divisions (BOX 2) of RPCs have revealed multipotency: in mice, rats and *Xenopus laevis*, clones containing only two cells (which is typical for a clone arising from a terminally dividing RPC) can be comprised of two very different cell types, such as a rod and an amacrine cell[6,12–14,17].

These clonal data demonstrated that RPCs are generally multipotent. However, these data could not determine whether the variability in clones was due to intrinsic differences among RPCs or extrinsic and/or stochastic effects on equivalent RPCs or their progeny. Furthermore, the fates identified within a clone demonstrated an RPC's 'potential' but not the ability of an RPC to make a specific cell type at a specific developmental time or its 'competence' (BOX 2). Moreover, although many genes that regulate the development of retinal cell types have been studied, using the now classical gain- and loss-of-function approaches[18,19], the precise roles of such regulators in defining an RPC's competence or potential have not been well elucidated, as most studies have examined the outcome of a perturbation on the development of a cell type but not the stage and/or cell type in which such a regulator might carry out its action.

In this article, I consider possible models of cell fate determination in the retina that involve intrinsic and extrinsic determinants. I discuss several lines of evidence that suggest that many RPCs undergoing terminal divisions are molecularly specified to produce particular retinal cell types. I also discuss how stochastic mechanisms may be involved in the mitotic patterns of RPCs and in the choice of cell fate, and consider how the temporal order of the birth of different cell types in the retina may be controlled.

Models of fate determination

On the basis of our current understanding of developmental mechanisms, we can consider both 'intrinsic' and 'extrinsic' models for the determination of cell fates (BOX 2; FIG. 2). As discussed further below, stochastic mechanisms may operate within each of these models[20–23].

One intrinsic model proposes that, as development unfolds, each RPC lineage progresses through an invariant series of competence states[24]. Within each state, the RPC's competence is defined by its ability to produce particular retinal cell types at that time. The entire temporal series of competency states is proposed to be achieved by an intrinsic programme (FIG. 2a). According to this model, variations in the size and complexity

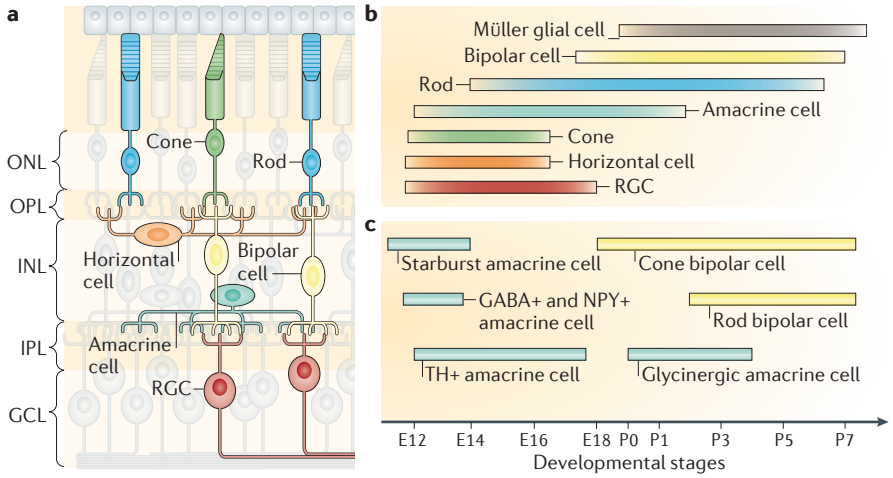

Figure 1 | **Retinal cell types and their birth order.** **a** | A cross-section of the retina. The retina is organized into three layers of cell bodies (the outer nuclear layer (ONL), inner nuclear layer (INL) and the ganglion cell layer (GCL)) and two layers of neuropil (the outer plexiform layer (OPL) and the inner plexiform layer (IPL)). Retinal neurons comprise primary sensory cells (rods and cones), interneurons (horizontal cells, bipolar cells and amacrine cells) and output neurons (retinal ganglion cells (RGCs)). There are many subtypes of each type of neuron that vary not only in terms of their functions and features but also in their frequencies[3,8]. Each cell type is distributed such that the entire retina has the full complement of cell types, and each subtype is evenly spaced or tiled across the retina. **b** | The birth dates of each of the major cell types in the rat and mouse retina are indicated[5,8]. Classical ^{3}H-thymidine birth dating has shown the same overall order of retinal cell birth dates across many species. **c** | The birth dates of different retinal cell subtypes are indicated. Birth dating carried out using bromodeoxyuridine or ethynyldeoxyuridine and retroviral marking has shown that amacrine and bipolar cell subtypes are born in a specific order[9–11]. Amacrine cells that use GABA as a neurotransmitter are born earlier than those that use glycine. Starburst amacrine cells use GABA as well as acetylcholine and are born very early, whereas GABAergic amacrine cells that also express neuropeptide (NPY) are born later, and tyrosine hydroxylase (TH)-expressing amacrine cells are born later still. Glycinergic amacrine cells are born primarily in the postnatal period. Cone bipolar cells are born throughout the period of bipolar cell genesis, and rod bipolar cells are born only in the later part of this period.

of the clones of labelled RPCs arise because an RPC does not produce postmitotic daughter cells in every competence state. The production of postmitotic daughter cells might be regulated by mechanisms that are somewhat independent of the competency states — for example, through Notch signalling[25,26] and/or stochastic mechanisms[22,23].

Another intrinsic model proposes that there are distinct types of RPCs that are established at an early stage of retinal development, each of which then goes through its own intrinsic programme to produce clones that have some specific types of progeny. This model does not imply that there is no overlap in the cell types generated in each lineage: indeed, it is likely that there would be overlap, particularly in the production of the more abundant cell types, such as photoreceptor cells (FIG. 2b). This model is similar in overall concept to that used in several areas of the *Drosophila melanogaster* nervous system, such as the ventral nerve cord and medulla[27]. For example, in the ventral nerve cord, positional cues arrayed along the anterior–posterior and dorsal–ventral axes define sets of neuroblasts, which are referred to as type I neuroblasts. Each type I neuroblast generates a clone containing specific cell types by producing, in each division, another neuroblast as well as a terminally dividing cell that is referred to as a ganglion mother cell (GMC). In order to generate even more complexity, within the central brain, type II neuroblasts produce a set of proliferative daughter cells, referred to as intermediate neural progenitors (INPs), which divide multiple times to produce larger and more complex clones (again containing defined cell types). GMCs also are produced by INPs. The products of GMC divisions are often asymmetrical, with Notch activity required to create this asymmetry[28].

At the other extreme, one can propose an extrinsic model in which all RPCs are equivalent at all times and so are competent to produce all retinal cell types. According to this model, all RPCs would produce equivalent postmitotic progeny and extrinsic cues would induce different fates in these progeny (FIG. 2c).

The results of studies that have examined the competence of RPCs in different environments have shed light on the viability of these intrinsic and extrinsic models. Such studies have shown that RPCs at any particular developmental stage can produce a limited repertoire of daughter cell types. Thus, early RPCs produce cells with early fates when transplanted into a heterologous[29] or late retinal environment[30], and late RPCs produce cells with late fates even when placed in an early environment[31]. Similarly, RPCs produced cells with temporally appropriate fates when isolated in culture[22,32–34], and heterochronic mixing experiments (in which cells derived from different developmental stages are co-cultured) also showed that intrinsic factors contribute to proliferation and the timing of the onset of differentiation[35,36]. These findings provide evidence for the models that suggest that there are intrinsic changes in states of competence within RPCs over time[24]. As described below, several additional lines of evidence support this hypothesis.

Heterogeneous RPC gene expression

The intrinsic models proposed above would suggest that there are differences in gene expression among RPCs, and these differences would be expected to relate to the competency states of these cells. The advent of methods to comprehensively assess gene expression has enabled the interrogation of the developing retina for temporal changes in gene expression[37,38]. This has included the analysis of gene expression in single RPCs and single newborn cells of many types[9,39–41]. These studies have demonstrated that RPCs are extremely heterogeneous.

Although many differences in gene expression within RPCs were found, few consistent trends that correlated with developmental age were seen in the single-cell profiles of RPCs. This inconsistency does not seem to be related to systematic irreproducibility of the techniques or to differences that would be expected to originate owing to the location of a cell in the retina and/or its position in the cell cycle. Indeed, *in situ* hybridization using more than 1,000 probes[9,37,39–42] and previous research using serial analysis of gene expression (SAGE), a method to digitally track mRNAs[43], to monitor temporal patterns of gene expression within RPCs[37] have demonstrated the validity of these single-cell microarray data. Nonetheless, it is possible that the variability in gene expression results from biological noise: that is, transcripts might be differentially expressed in different cells if the regulation of transcription is imprecise. Such

Box 1 | **A case for ontogeny recapitulating phylogeny**

The evolution of the eye has been a topic of great interest for hundreds of years, and an excellent overview and analysis of this topic are provided by recent reviews[123,124]. Here, the possibility that the birth order of retinal neurons is due to the order in which they arose in evolution is considered.

The earliest cell types generated in extant retinae are photoreceptor cells (cones), horizontal cells and retinal ganglion cells (RGCs) (FIG. 1b). Similarly, during evolution, the earliest photoreceptive tissue was probably composed of photoreceptors alone. The later evolution of output cells, such as RGCs, may have enabled the transmission of processed information to one or multiple locations. Alternatively (or additionally), early RGCs might have transduced light signals using their own photosensitive pigment, melanopsin[125]. As more cell types evolved in the retina, circuits that extract additional features of the visual scene could have been added. It is likely that photoreceptors in primitive retinae directly contacted output cells, with the horizontal cells providing inhibition. One can see remnants of this proposed primitive stage in the contacts between newborn photoreceptors and RGCs in the developing ferret retina[126]. Moreover, even in extant retinae, horizontal cells initially reside adjacent to RGCs[127–129] before migrating to their position near photoreceptors (FIG. 1a). It is thus perhaps not unexpected that cones and horizontal cells have been shown to be the progeny of the same RPC (with an additional earlier division of this RPC producing an RGC[55]). Rods, which evolved later, might then not be derived from the same early RPC from which cones are derived.

Amacrine cells are very similar to horizontal cells: some of the same regulators are involved in their development[114] and it has been suggested that they may have a common precursor during evolution[130]. Alternatively, amacrine cells may have evolved at a later stage. Subtypes of amacrine cells may have evolved from an early amacrine cell, or from a common amacrine and horizontal cell precursor, as particular circuits evolved. One circuit that is likely to have evolved early computes the direction of motion[131]. The starburst amacrine cell, which makes direct connections with direction-selective RGCs[132], is among the earliest born of the amacrine cell subtypes[11]. An early origin of direction-selective circuits may also explain why RPCs that are biased towards the production of a particular type of direction-selective RGC appear early in retinal development[67].

Further evidence that birth order may reflect evolutionary order comes from the observation that cone opsins evolved before rod opsin[133], and cones are generally born before rods (FIG. 1b). Similarly, cone bipolar cells are born before rod bipolar cells (FIG. 1c), and the rod circuitry uses the cone circuitry for the transmission of rod signals[134].

All RPCs may be competent to produce photoreceptors: many retinal clones contain photoreceptors[6,12–15,17], and photoreceptors are lost when either PAX6 or retina and anterior neural fold homeobox (RAX), which are both expressed by all RPCs, is removed after the optic cup forms[135,136]. Notch, a protein that evolved early[137], inhibits photoreceptor determination[26,138] and thus may have been involved in the generation of a pool of cells that could evolve into non-photoreceptor fates in the early retina. Downstream of Notch, some diversification of cell fates was accomplished through the evolution of the basic helix-loop-helix genes[139]. Diversification also occurs through the action of homeobox genes, including *Rax*, *Pax6*, orthodenticle homologue 2 (*Otx2*), cone-rod homeobox (*Crx*), visual system homeobox 1 (*Vsx1*) and *Vsx2* (REF. 18). As all of these encode the paired type of homeobox, many extant retinal genes may have retained regulatory sequences that were derived from the networks that used *Pax6* and/or *Rax* in the early photoreceptor cells.

The latest-born cell types — rods, bipolar cells and Müller glia — probably arose late in evolution[123]. Bipolar cells may be sister cells of photoreceptor cells: they share expression of some transcription factors and aspects of their molecular transduction mechanisms, and both possess an unusual ribbon synapse. The functions ascribed to Müller glia[140] may have evolved as the retina became more complex, and thicker, requiring structural, nutritional, metabolic and trophic support.

differential transcription could be considered to be biological noise, as it may not be relevant to the development of the retina.

Lineage studies have been carried out to determine whether differences in the expression of a particular mRNA are correlated with the number or types of daughter cells produced by RPCs (see below). To shed some light on the myriad gene expression patterns seen in RPCs, it would also be helpful to carry out single-cell profiling on a much larger number of RPCs than has been profiled to date. This might yield a more robust classification scheme for discrete classes of RPCs, if such classes exist. Alternatively, it might reveal that there are no discrete classes but that there is a continuum of gene expression along the temporal axis.

Biased terminal divisions of RPCs

If the clone of a labelled RPC is observed to contain only 1–4 cells, one can infer that the originally marked RPC, and/or its immediate progeny, produced only postmitotic cells. Studies using Cre fate mapping in the mouse (in which the expression of Cre recombinase, and thus of a detectable marker, is restricted to cells expressing a particular gene), retroviral labelling of mouse and chick RPCs and live imaging in zebrafish have provided evidence for the existence of RPCs expressing particular molecular markers that undergo terminal divisions to produce specific types of progeny, in line with the intrinsic models described above.

Cre fate mapping in the mouse. Several studies have examined the fate of cells labelled by the expression of Cre under the control of the promoter of atonal homologue 7 (*Atoh7*; also known as *Math5*), a basic helix-loop-helix (bHLH) gene that is expressed primarily early in development[44–46]. These studies showed that many early-born retinal cell types, and a few of the late-born cell types, either expressed *Atoh7* themselves or were derived from RPCs that expressed this gene. As only a few late-born cell types were labelled, it is likely that the *Atoh7*-expressing cells were mostly postmitotic at the time of labelling or were early RPCs that were close to undergoing their terminal divisions[44].

Examination of cells with a history of expression of two other bHLH genes, achaete-scute complex homologue 1 (*Ascl1*) or neurogenin 2 (*Ngn2*; also known as *Neurog2*), has also been carried out using Cre fate mapping[47]. In this study, labelling was seen primarily in small clusters of cells or single cells, again suggesting that these bHLH gene-expressing cells must have been either postmitotic or terminally dividing RPCs. Cells expressing — or derived from cells expressing — *Ascl1* and *Ngn2* included those from all major retinal cell classes. However, retinal ganglion cells (RGCs) were rarely labelled in the *Ascl1*-derived lineages, whereas they were labelled more often in the *Ngn2*-derived lineages. It will be important to distinguish cells that express these bHLH genes in the postmitotic state from those that derive from RPCs that express these genes. Nonetheless, these studies suggest that the expression of these bHLH genes varies among RPCs in a manner that is partially correlated with the different types of daughter cells that they produce. They further suggest that most of the RPCs that express these bHLH genes undergo terminal division.

Retroviral clonal analysis in the mouse. Early retroviral lineage studies used retroviruses that could infect any type of RPC[13,17]. A newer version of this method, in which mouse genetics is used to drive the expression of an avian retrovirus receptor, TVA[48], allows the progeny of specific RPCs to be traced. By expressing

Box 2 | **Key definitions in retinal progenitor cell lineage analysis**

- Retinal progenitor cell (RPC): a mitotic cell in the retina.
- Clone: a clone is comprised of all of the progeny from a single RPC. In lineage studies that define clones, infection with a retrovirus, injection of a tracer or high-resolution imaging of cells expressing a reporter protein are used to follow the cell divisions and progeny of individual mitotic cells.
- Terminally dividing RPC: an RPC that divides to produce two postmitotic daughter cells.
- Intrinsic determinants: molecules or pathways present within an RPC that regulate its behaviour and/or that are inherited by a daughter cell and influence its fate. One example of an intrinsic determinant is a transcription factor or microRNA that is inherited by a daughter cell and biases its fate. Intrinsic determinants might be asymmetrically inherited during cell division, resulting in two different fates for the daughter cells.
- Extrinsic determinants: signals — which may be soluble or cell associated — in the extracellular environment of a cell that influence its behaviour.
- Stochastic event: an event that is determined by probability (that is, it is not rigidly determined). It is important to note that stochastic does not mean 'unbiased', and developmental events can be both biased and stochastic[20]. For example, determinants of different fates may be expressed by an RPC, with the levels of the determinants for one particular fate being higher than those for other fates. These determinants might be inherited stochastically, so that each daughter cell is more likely (biased) to inherit the more abundant determinants.
- Competence: the ability of a cell to produce a particular type of daughter cell in a short time frame. As it divides and moves through different states of competence, an RPC may be competent to generate only one or a few types of cells at any one moment in time, in keeping with the birth order of retinal neurons. If an RPC that is in a particular state of competence does not produce a postmitotic daughter cell, one may not be able to detect evidence of its competence.
- Potential: the potential of an RPC is defined by all of the types of cells it, or its progeny RPCs, can produce during the course of retinal development. The cells present at the earliest stages of optic vesicle development are probably able to generate all retinal cell types and may thus be 'totipotent'. However, when one places one of these early RPCs in a late environment and tests whether it can produce a late-born cell type, it does not, as it is not in a state of competence that would allow it to do so. However, after some number of cell divisions and/or the passage of time, later RPCs will have the competence to produce late cell types.
- Determined: an RPC is said to be 'determined' if it produces an invariant set of daughter cell types even when placed in different environments. RPCs that reproducibly give rise to specific progeny are often inferred to be determined, even when they are not challenged by different environments.
- Specified: RPCs or newly postmitotic cells that are biased towards a particular fate, but which are not determined, are said to be 'specified'. This can be evidenced by the expression of markers of a fate, which in some instances might be labile.

TVA only in cells that express a particular gene, the clonal progeny of those cells can be defined by infection using a gamma-retrovirus that is targeted to TVA-expressing cells[49]. As only mitotic cells can be infected with gamma-retroviruses[50], this method eliminates the ambiguity that arises in Cre fate-mapping studies regarding whether a cell was marked when it was postmitotic or whether it derived from an RPC that expressed Cre.

Oligodendrocyte transcription factor 2 (*Olig2*) is a bHLH gene that is expressed in a subset of RPCs and in a subset of differentiating bipolar cells[41,51,52]. By using a knock-in mouse strain in which TVA is expressed from the *Olig2* promoter (*Olig2*-TVA strain)[53], the types of clones that derive from *Olig2*-expressing RPCs were defined[51] (TABLE 1). When infection was initiated early, at embryonic day 13.5 (E13.5)–E14.5, *Olig2*-derived clones contained only one or two cells, indicating that *Olig2*-expressing RPCs were terminally dividing or, in rare cases, that *Olig2* was expressed one cell division earlier than the terminal division. This stands in contrast to the clones labelled by infection with a control retrovirus (one that can infect any type of RPC), which contained an average of about 30 cells. The clones derived from RPCs expressing *Olig2*-TVA almost exclusively contained cones and horizontal cells (FIG. 3a). RGCs, which are also produced at E13.5–E14.5, were not seen among the *Olig2*-derived progeny. Cones and horizontal cells are two of the rarest cell types in the retina[8], so this was a very significant skew in the cell types produced. It is not clear whether all cones and horizontal cells arise from an *Olig2* lineage; however, conventional Cre fate mapping suggests that the majority of cones and horizontal cells have a history of *Olig2* expression[51].

Clones that were derived from *Olig2*-expressing RPCs labelled late in development, at postnatal day 0 (P0) or P3, were also analysed[51] (TABLE 1). These clones were also small and showed a skew in the types of cells produced; they were comprised almost entirely of rods, with some amacrine cells. Almost no Müller glia and very few bipolar cells were labelled. The paucity of bipolar cells produced by *Olig2*-expressing RPCs is interesting, as many postmitotic bipolar cells express *Olig2* during their differentiation[52]. This discordance illustrates the importance of determining whether a cell that is labelled by Cre fate mapping is labelled as a result of Cre activity in its progenitor cell or Cre activity after cell cycle exit.

Live imaging in zebrafish. Cone and horizontal cell lineages were studied in the zebrafish retina using live imaging. Horizontal cells were shown to be produced by an unusual RPC that only made horizontal cells and underwent mitosis in the middle of the retinal layers rather than at the apical surface[54] (TABLE 1). This RPC was marked by the expression of a reporter for *connexin 55.5*, a horizontal cell marker, as well as by reporters for *prospero homeobox 1a* and *pancreas specific transcription factor 1a*. It was estimated that most, if not all, horizontal cells are produced by this non-apical RPC[54].

A more recent study imaged RPCs expressing a reporter based on *thyroid hormone receptor-β* (*thrb*; also known as *nr1a2*)[55] (TABLE 1), a marker for cones that express the long-wavelength opsin (L cones). L cones were the predominant cell type produced by symmetrical terminal divisions of *thrb*-expressing RPCs. Furthermore, divisions that produced four-cell clones from a *thrb*-expressing RPC were observed. One such clone comprised two L cones and two horizontal cells, each made by symmetrical terminal divisions. Additional types of divisions were observed in which an RGC and an RPC that produced L cones were made, and there was one example of a clone made by two L cone-producing RPCs: that is, a clone consisting of four L cones.

A reporter based on *cone-rod homeobox* (*crx*), which is expressed in RPCs, rods, cones and bipolar cells, also showed homotypic patterns of cell production from terminal divisions (FIG. 3b; TABLE 1). RPCs expressing *crx* produced pairs of cones that expressed the same opsin type (that is, the medium-wavelength (M), short-wavelength (S), L or ultraviolet (UV)-wavelength cone opsins). The production of homotypic pairs of cones

expressing the same opsin types by terminal divisions stands in contrast to observations of opsin choice in some other species. In the cones of Old World primates, L versus M opsin choice is determined by which opsin gene captures the activity of a single genomic locus control region, which leads to a random pattern[56–58]. This type of regulation probably reflects the recent duplication of these genes in this lineage. In the *D. melanogaster* retina, yellow and pale ommatidia result from a stochastic mechanism originating in the R7 photoreceptor cell, which then signals to the R8 photoreceptor cell to produce a specific type of opsin[59].

Live imaging of zebrafish divisions was carried out in which RPCs that expressed *ath5* (equivalent to mouse *Atoh7*) were marked by expression of a green fluorescent protein. The *ath5*-expressing RPCs appeared to produce small clones. Long-term imaging to follow the fates of all daughter cells was not possible[60]. However, the authors reported that there were many asymmetrical divisions that produced at least one RGC. Another live-imaging study of randomly labelled zebrafish RPCs also examined the divisions that produced RGCs[23]. They found that most RGCs are produced by asymmetrical divisions that produce one RGC and one RPC, and that this pattern of production was more frequent than would be predicted by chance. This study also reported that there were more homotypic pairs of photoreceptors, horizontal cells, amacrine cells and bipolar cells produced in terminal divisions than would be predicted by chance.

Retroviral marking in the chick. The clones produced by retroviral marking of early RPCs in the chick can be large and complex[15,61]. Although analysis of the entire cellular composition of such large clones has not been carried out owing to the very high density of cells in such clones, horizontal cells were found to be relatively easy to quantify, as they were segregated from other cells and were large cells with obvious morphological features. The chick retina contains three types of horizontal cells, H1, H2 and H3, which vary in their morphology and in their patterns of connectivity to rods and cones[62,63]. The retroviral marking studies showed that some intermediate-sized clones contained only one or two horizontal cells. In the clones containing two horizontal cells, these were homotypic pairs: either a pair of H1 cells or a pair of H3 cells. Two clones were seen with only two cells in total, both of which were H1 cells. Interestingly, H2 cells

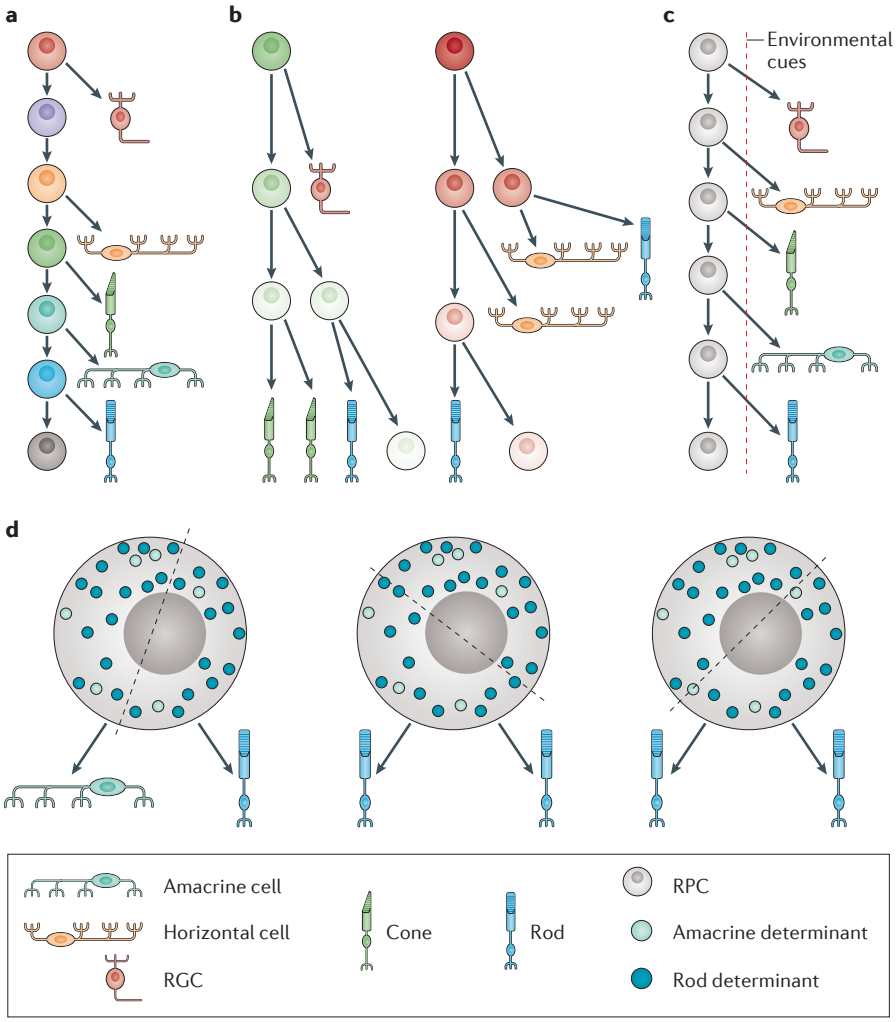

Figure 2 | **Models of retinal cell fate determination.** Several models for the determination of retinal cell types are depicted. Combinations of these models can also be considered and may apply to particular periods of time and/or for particular fates in different species. Intrinsic programmes that run in a temporal order may operate in retinal progenitor cells (RPCs). **a** | According to one intrinsic model, all RPCs follow a single, intrinsic temporal order of competency states (represented here by different colours), during which they will be capable of producing particular types of daughter cells (for simplicity, only one of the possible daughter cell types is shown at each stage). As they traverse these states, however, every RPC will not make every type of cell that it is competent to produce, as postmitotic daughter cells will not be made during each state of competence (for example, the purple RPC does not make a postmitotic daughter cell). The final type of RPC (grey) may develop the functions of Müller glia[42,141] and serve as a stem cell in some species[142]. **b** | According to another intrinsic model, there may be patterning among early RPCs that leads to biases in the production of specific types, or subtypes, of cells. This is illustrated as two lineages originating with green or red RPCs. All RPCs, however, are predicted to retain the competence to produce rods and cones. In the hypothetical examples shown, clones derived from the green RPC would be enriched with cones, whereas clones derived from the red RPC would be enriched with amacrine cells. **c** | An extrinsic model for the production of retinal cell types suggests that all RPCs are equivalent in terms of their competence to produce all retinal cell types. Newly postmitotic daughter cells would encounter environmental cues, which would direct their choice of cell fate. **d** | The stochastic distribution of determinants during cell division can occur within RPCs that have intrinsic biases towards certain fates, which may generate heterogeneity in clones. A terminally dividing RPC that is intrinsically biased towards the production of rods and amacrine cells is shown. As this RPC has a much higher level of rod determinants than amacrine determinants, each daughter cell will be more likely to become a rod than an amacrine cell. Three types of two-cell clones could be produced, with the frequency of each clone type being dictated by the relative levels of the determinants and the distribution of determinants to each daughter cell. Most division planes (dashed lines) would distribute a subthreshold level of amacrine determinants and thus most daughter cells would be rods, with only a rare clone comprising two amacrine cells (not shown).

Table 1 | **Specific types of retinal progenitor cells can produce restricted types of progeny**

Organism (age)	Method of marking	Type of RPC	Progeny or clone composition	Type of division	Refs
Zebrafish	Live imaging	Express reporters for *cx55.5*, *prox1a* and/or *ptf1a*	HC only	Terminal	54
Chick (E3–E5)	Retrovirus	Random	Homotypic pairs of HCs type 1 or 3	Terminal	61
Chick (E5–E7)	Retrovirus	Express reporter for *THRB*	Cones and HCs	Terminal	64
Mouse (P0, P3)	Retrovirus	*Olig2*-expressing	Homotypic pairs of rods or heterotypic pairs of rod and AC	Terminal	51
Mouse (E13, E14)	Retrovirus	*Olig2*-expressing	Homotypic pairs of cones, HCs or heterotypic pair of HC and cone	Terminal	51
Mouse (E8–E12)	Cre	*Cdh6*-expressing	*Cdh6*-expressing RGCs and multiple other cell types	Asymmetrical	67
Zebrafish	Live imaging	Express reporter for *thrb*	Homotypic pairs of L cones or homotypic pairs of HCs	Terminal	55
Zebrafish	Live imaging	Express reporter for *thrb*	Homotypic pairs of HCs and homotypic pairs of L cones, and/or an RGC	Terminal and penultimate divisions	55
Zebrafish	Live imaging	Express reporter for *crx*	Homotypic pairs of L, S, M or UV cones	Terminal	55

Various lineage tracing methods were used to follow the fate of progeny of specific types of retinal progenitor cells (RPCs). A terminal division is one in which both progeny exit the cell cycle, whereas the penultimate division refers to the division preceding the terminal division. AC, amacrine cell; *Cdh6*, cadherin 6; *crx*, *cone-rod homeobox*; *cx55.5*, *connexin 55.5*; E, embryonic day; HC, horizontal cell; L, long-wavelength; M, medium-wavelength; *Olig2*, oligodendrocyte transcription factor 2; P, postnatal day; *prox1a*, *prospero homeobox 1a*; *ptf1a*, *pancreas transcription factor 1a*; RGC, retinal ganglion cell; S, short-wavelength; *THRB*, thyroid hormone receptor-β; UV, ultraviolet.

were not seen in homotypic pairs. H2 cells were, however, over-represented among the clones that contained only one horizontal cell. The homotypic H1 and H3 clones were proposed to be generated by terminal divisions of a determined H1 or H3 RPC (FIG. 3c; TABLE 1). Based on the reported behaviour of *THRB* reporter-expressing cells discussed below, it is likely that an H2 cell is produced in a terminal division, with a cone as its sibling[64].

A *THRB* reporter was also used to study the genesis of cones in the chick[64]. A 40 bp highly conserved region of *THRB* (*THRBCRM1*) was found to be expressed in an interesting pattern in cells other than cones, as was the aforementioned *thrb* reporter in zebrafish[55] and a *Thrb* reporter (*ThrbICR*) in mice[65]. The cell types that expressed chick *THRBCRM1* were identified to be a subset of RPCs, cones and developing horizontal cells. These patterns were further explored to determine whether they were related by lineage. A retroviral analysis of the progeny of RPCs expressing *THRBCRM1* showed that their progeny were horizontal cells and photoreceptors.

These findings suggest that many RPCs that undergo terminal divisions and produce specific pairs of daughter cells are almost certainly patterned by intrinsic cues. The intrinsic information inherited by the postmitotic daughter cells heavily biases their fates. As discussed further below, each fate may be specified but is not always rigidly determined: at least in some cases, feedback inhibition is able to alter some fates.

Bias in non-terminal divisions

The data cited above show that there are terminally dividing RPCs that produce specific types of daughter cells. In some of these studies, there was also evidence for bias in the divisions taking place one division earlier than the terminal division. For example, some of the *thrb*-expressing RPCs imaged in zebrafish produced clones of three or four cells, which included only L cones (not M, S or UV cones), RGCs and/or horizontal cells[55] (TABLE 1). Another recent study using Cre fate mapping in the mouse also provides evidence of patterning in RPCs that is most likely to occur prior to a terminal division. It has been shown that different subtypes of ON–OFF direction-selective RGCs (ooDSRGCs) differ in the expression of certain classes of adhesion molecules[66]. The ooDSRGCs that respond to upward motion express cadherin 6 (CDH6), as do a subset of early RPCs[67]. Intriguingly, it was shown that *Cdh6*-positive RPCs produced clones in which the RGC (or RGCs) produced were heavily biased towards those expressing *Cdh6* (REF. 67) (FIG. 3d; TABLE 1). *Cdh6*-expressing RGCs were not seen when RPCs and their clones were labelled using a marker of another type of RGC. Evidence that *Cdh6*-expressing RPCs were not terminally dividing was provided by the fact that the clones consisted of more than one or two cells. Photoreceptors, Müller glia and multiple types of bipolar and amacrine cells were also present in these clones, without any obvious bias in the subtypes of these cells. However, examination of a larger set of clones using a more complete set of markers for the many retinal subtypes is needed to rule out the possibility that there is a skewed distribution of other types of cells within these clones.

In the study described above, *Cdh6*-expressing cells were marked by the insertion of the gene encoding a tamoxifen-inducible form of Cre into the *Cdh6* locus. Injection of tamoxifen at E8 and later, but not at E7, led to the generation of marked clones. The production of the first RGCs occurs at least 2 days after E8 (REF. 68). As it is estimated that the completion of the cell cycle takes 10 hours at this developmental stage[69], at least a few cell cycles would take place between the tamoxifen injection at E8 and the terminal division of an RPC that produces *Cdh6*-expressing RGCs. Exactly how many cell cycles would separate these events is ambiguous owing to the unknown kinetics of the induction of Cre activity and the recombination event, but tamoxifen-induced recombination has been estimated to occur within 24 hours in most tissues[70]. Although direct examination of the kinetics of the production of *Cdh6*-expressing RGCs is required to confirm this notion, these observations indicate that the early retina contains RPCs that are patterned early to produce a specific subtype of cell, and it is likely that the effect of this patterning is seen up to several cell cycles later.

Other studies have examined the distributions of specific subtypes of cells in larger clones. As described above, one study in the chick examined horizontal cell subtypes in intermediate-sized clones that contained only one or two horizontal cells[61]. An analysis of

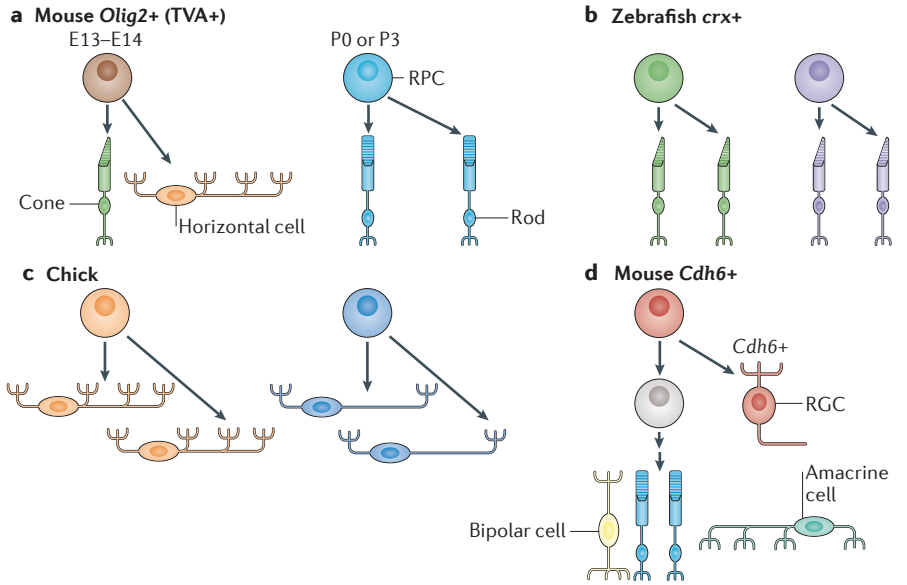

Figure 3 | **Bias in the types of neurons produced by specific RPCs. a** | The terminal divisions of oligodendrocyte transcription factor 2 (*Olig2*)-expressing retinal progenitor cells (RPCs) produce specific types of progeny, which differ over time. The avian virus receptor, TVA, was knocked into the *Olig2* locus in transgenic mice. Infection of TVA-expressing cells with a gamma-retrovirus that infects only mitotic TVA-expressing cells was used to assess the fates of cells derived from *Olig2*-expressing RPCs. The *Olig2*-expressing RPCs made terminal divisions, as clones consisted of only one or two cells. The clones had restricted fates, with different fates produced by *Olig2*-expressing RPCs at different ages[51] (TABLE 1). *Olig2*-expressing RPCs labelled at embryonic day 13 (E13)–E14 almost exclusively produced cones and horizontal cells, whereas clones generated by *Olig2*-expressing RPCs at postnatal day 0 (P0) or P3 contained mainly rods and a few amacrine cells. **b** | Live imaging of zebrafish RPCs expressing a reporter for *cone-rod homeobox* (*crx*) showed that these cells produce homotypic pairs of cones that express a particular opsin type (shown here are the medium (green) and ultraviolet (UV, purple) opsin)[55]. **c** | Retroviral labelling of the E3–E5 chick retina resulted in large clones, some of which contained only one or two horizontal cells[61]. The pairs of horizontal cells were almost always homotypic pairs of H1 cells (orange) or H3 cells (blue), and it was proposed that they arise from terminal divisions of determined RPCs. Clones that contained only a single horizontal cell (not shown) typically contained an H2 cell, the sibling of which was likely to be a cone[64]. **d** | Clones from cadherin 6 (*Cdh6*)-expressing RPCs show a bias in the production of retinal ganglion cells (RGCs) that express *Cdh6* (REF. 67). A tamoxifen-inducible form of Cre recombinase was knocked into the *Cdh6* locus in mice. *Cdh6* is expressed in a type of ON–OFF direction-selective RGCs (ooDSRGCs) as well as in a subset of embryonic RPCs. Clones labelled with Cre under the control of low doses of tamoxifen comprised many cell types, including RGCs. Of these, there was a significant bias towards *Cdh6*-expressing ooDSRGCs. Other cell types, including amacrine and bipolar cells, were also observed in these clones, with no significant skew towards particular subtypes.

the very large clones labelled in this study shows that 7 out of 10 of the clones that contained >100 horizontal cells also had a very skewed distribution of horizontal cell subtypes (C.C., unpublished observations) (Supplementary information S1 (table)). These skewed distributions did not seem to be due to the location of the large clones, as analysis of the distribution of horizontal cell subtypes showed that it is fairly even across the retina. An intriguing study in *X. laevis* also found evidence of bias towards particular subtypes of cells when marking was initiated very early in blastomeres[71,72]. Remarkably, there were statistically significant differences in the numbers of different subtypes of amacrine cells labelled when different blastomeres were marked. Currently, it is unclear how we should interpret the findings of skewed distributions in the final output of RPCs marked so early in development. However, we can speculate that they may reflect the scenario outlined in FIG. 2b, in which early RPCs are biased towards certain fates.

The mechanism (or mechanisms) by which very early events can lead to the genesis of specific cell types later in development has been determined for the ASE neurons, a pair of sensory neurons in *Caenorhabditis elegans*[73]. In the very early stages of the lineage that produces one of the ASE neurons, the 'priming' of the chromatin state of a particular microRNA (miRNA) is induced by Notch signalling. This priming has a later impact on gene expression in the differentiating neurons that inherit the miRNA. The events that might account for the bias of early RPCs have not been investigated, but miRNAs have been shown to play a part in the birth order of different retinal cell types[74–78], as discussed below. Inheritance of a 'primed' chromatin state would be another mechanism that might link early and late events, and this possibility warrants further examination.

Specifying daughter cell fates

The observations described above demonstrate that specific daughter cell types are produced by marked RPCs. These findings call for a molecular explanation. There have been many studies of the roles of individual transcription factors or signalling pathways in the specification of retinal cell fates[18,19]. A common outcome of studies in which loss of function of a factor is engineered is that not one but multiple fates are affected. In a similar vein, most gain-of-function studies lead to less-than-complete penetrance: for example, the addition of a transcription factor does not commit all cells to one fate. These findings have led to the consensus that the context of a cell determines its response to a particular perturbation[79–81]. The context might be determined by many types of regulatory factors, including a chromatin state, miRNAs, transcription factors and the state of signalling pathways. Definition of the factors within a particular type of RPC that create context, or more generally bias the fate of its progeny, will thus need to be addressed.

One can first ask whether the markers that define different types of terminally dividing RPCs also control the fates of their daughter cells. OLIG2, for example, is not a major contributor to the fate decisions of the daughter cells of *Olig2*-expressing RPCs because loss of OLIG2 does not produce any obvious retinal phenotype, although a thorough analysis of mutant retinae was precluded owing to neonatal lethality[51]. By contrast, the results of studies in the spinal cord showed its role in fate determination[82]. Other gain- and loss-of-function studies of bHLH genes have shown that these genes are heavily interconnected in a network, with redundancy and compensation making it difficult to discern their precise roles[83–86]. These effects may hide the role of OLIG2 in the retina.

CDH6 is an adhesion molecule that is expressed in a specific type of RGC that also expresses CDH3. Mice in which CDH6 was absent retained *Cdh3*-expressing RGCs, but there were defects in the targeting of their

Glossary

Direction-selective RGCs
(Direction-selective retinal ganglion cells). Cells that fire action potentials in response to motion in particular directions within the visual field.

Ommatidia
The basic unit of the retina of some invertebrates, consisting of a repeated pattern of photoreceptor cells that express particular opsin genes and have specific projection patterns into the brain.

Opsin
A membrane-bound G protein-coupled receptor, which is found in rod and cone photoreceptors, that initiates phototransduction. Its spectral sensitivity depends on the sequence of amino acids that tune the activity of the small chromophore, 11-*cis*-retinal.

axons to some retinorecipient areas in the brain, probably owing to a lack of target recognition[87]. An examination of the other features of those RGCs was not reported. In zebrafish, a decrease in the levels of Cdh6 led to a reduction in proliferation, alterations in the expression of molecules involved in the Notch pathway and changes in the differentiation of several cell types[88]. However, it is not clear whether zebrafish express Cdh6 in a subset of RPCs or how to relate these findings to those in the mouse.

THRβ, which is expressed in a subset of RPCs that produce particular types of cells, including cones (see above), does have a role in cone development but not in the determination of cones. In zebrafish, loss of Thrβ leads to the loss of L opsin expression and a gain of UV opsin expression[55]. In mice, which only have an M and an S opsin, loss of THRβ leads to a loss of M opsin and a gain of S opsin expression[89]. As many cones in the mouse express both M and S opsin[90], it is likely that these effects of THRβ are on opsin regulation but not on cone identity[91–93].

The context of the RPCs that produce cones and horizontal cells has been explored and compared with that of the late RPCs that produce rods but not cones or horizontal cells[64]. The RPCs that produce cones and horizontal cells (that is, those that express *ThrbCRM1*) were found to express orthodenticle homologue 2 (*Otx2*) and one cut domain family member 1 (*Oc1*), whereas the late rod-producing RPCs do not express *Oc1* but do express *Otx2*. Loss of OTX2 had previously been shown to lead to a severe reduction in the formation of rods, cones, horizontal cells and bipolar cells[94,95]. Gain- and loss-of-function studies of *OC1* in mice and chicks showed that the presence or absence of OC1 defines the context in which OTX2 works to determine a cone versus a rod.

The aforementioned studies suggest that factors working within specific types of RPCs, some of which are probably inherited by newly postmitotic progeny, can greatly bias the fate of the progeny. It will be important to define the contexts of RPCs and their progeny so that we can learn how the context allows for different outcomes in different cells, such as the different responses seen to the addition of a single transcription factor. The many factors that have already been defined that affect retinal cell fates should be examined with respect to this question, particularly in the molecularly defined RPCs. In addition, molecularly defined RPCs can be examined (for example, by RNA sequencing) to determine the differences that contribute to the specific fates of their daughter cells.

Influence of stochastic mechanisms

In addition to the intrinsic and extrinsic cues that are hypothesized to influence the choice of retinal cell fates, one can invoke stochastic mechanisms[20,22,23]. For example, according to one intrinsic model, there may be a distinct type of RPC that is competent to make a rod and an amacrine cell (FIG. 2d). Its progeny may receive differing amounts of rod or amacrine cell determinants, with stochastic mechanisms determining their distribution in the two daughter cells. Numb, which is distributed asymmetrically in divisions of late RPCs and which presumably acts intrinsically, is required to establish such asymmetrical fates[96]. Cross-inhibition among transcription factors is another intrinsic process that can occur in a stochastic manner to determine cell fate, as is the case in the vertebrate spinal cord[97] and in several invertebrate lineages[98]. Stochasticity can also play a part when extrinsic cues are important. A live-imaging study in the zebrafish spinal cord showed that extrinsic cues are not rigidly controlled in terms of their spatial distribution[99]. Several studies of retinal development have probed this issue of stochasticity with respect to RPC cell division patterns and cell fate choices.

Live imaging was used to monitor the production of clones by late embryonic rat RPCs using cultures of cells plated at very low density[22,33]. The recorded cell division patterns — that is, the production of mitotic and/or postmitotic daughter cells in a given division or a series of divisions — were shown to fit a stochastic model. The cell types produced by each division were not produced in a rigid order. The composition of the clones also fit a stochastic model: the final compositions of the clones reflected the abundance of each cell type within the data set, with a few exceptions. In addition to providing evidence suggesting that stochastic processes govern the production of these clones, live imaging suggested that the probabilities of producing the different cell fates were governed by intrinsic processes. This conclusion arose from the observation that there was little opportunity for cell–cell interactions among non-clonally related cells given the very low plating density of the cells. A live-imaging study of the zebrafish retina reached a similar conclusion regarding stochastic processes governing cell division patterns[23]. Here, clone growth was well predicted by a stochastic model of cell divisions. This study also found that the birth order of cells within a lineage did not rigidly follow the population-wide birth order. However, certain temporal rules were not violated: for example, very late-born cells (such as Müller glia) were not born before very early cells (such as RGCs).

The issue of stochasticity and cell fate choices can also be considered in light of the data from clonal analyses conducted *in vivo* using retroviruses in the postnatal rat, in which a large collection of more than 1,000 clones were recorded[13]. Clones containing only two cells were abundant in this data set and were almost entirely comprised of rods in combination with each of the postnatally generated cell types. The frequency of each of the types of two-cell clones was found to be as predicted by random associations of the four cell types, taking into consideration the abundance of each cell type in the pool of two-cell clones (Supplementary information S2 (figure)). The results of this analysis are consistent with a stochastic model in which a single type of late RPC with certain biases towards each of the four cell types produces the distribution of clone types (Supplementary information S2 (figure, part a)).

However, there is an alternative interpretation for the two-cell clone data from the rat (Supplementary information S2 (figure, part b)). The terminally dividing RPC pool might be heterogeneous, comprising distinct types of RPCs, each of which is heavily biased towards the production of a distinct pair of daughter cells. According to this model, there would be a large proportion of postnatal RPCs that are determined to produce only rods in a terminal division, as clones containing two rods comprise the majority of the two-cell clones. In addition, there would be a smaller pool of distinct RPCs specified for the production of a rod and a bipolar cell, another specified for the production of a rod and an amacrine cell and another specified for the production of a rod and a Müller glial cell. The pool might be even more heterogeneous, with biases towards different subtypes of

bipolar cells and amacrine cells, which would be consistent with the aforementioned types of terminally dividing RPCs that produce a pair of H1 or a pair of H3 cells in the early chick retina.

The *Olig2* lineage tracing study described above provides strong support for the concept of intrinsic differences that limit cell fate choices from terminal divisions and so argues against the existence of a single type of late RPC. However, among *Olig2*-expressing RPCs, there may be a stochastic choice to produce rod–rod or rod–amacrine cell clones (FIG. 2d) but not bipolar cells or Müller glia (TABLE 1). Arguing against this latter possibility is evidence from single-cell profiling and immunohistochemistry, which show multiple differences among *Olig2*-expressing cells, even at the same time point in development[41,47,51]. Nonetheless, it is possible that there is a stochastic aspect to the production of RPCs that express *Olig2*. It will be necessary to understand the processes that take place in the cell divisions further up the lineage tree of *Olig2*-expresssing cells as well as to probe the contributions of different gene expression patterns within terminally dividing *Olig2*-expressing cells.

One other aspect of note concerning the two-cell clones from rats and mice is that they are different from those of the zebrafish[23]. In zebrafish, bipolar cells and amacrine cells are produced as homotypic pairs from terminal divisions of RPCs, whereas in rodents these cell types are almost always paired with a rod[13,17] (Supplementary information S2 (figure, part a)). This probably reflects the high abundance of rods in the rodent retina. The development of a rod-dominant retina may have been enabled by a high degree of proliferation of late, rod-competent RPCs[100] and/or the addition of rod competence to many types of RPCs that had evolved earlier to produce other cell types. One other way to generate a large proportion of rods is to create a rod-restricted progenitor that is not a terminally dividing RPC. Rod-only clones of more than two cells are observed in the rat and mouse data sets[13,17], although a thorough analysis of their frequency relative to different models has not been carried out.

What drives temporal progression?

Temporal order in the genesis of neuronal cell types (FIG. 1b,c) is a conserved feature not only within the retina but within other areas of the CNS of vertebrates and invertebrates[101]. The mechanisms that drive the temporal progression in *D. melanogaster* are being determined and involve transcription factor networks, miRNAs and protein stability[27]. In the retina, miRNAs have been identified as having a role, as revealed by studies in *X. laevis* and mice.

The genesis of bipolar cells late in retinal development requires two transcription factors, OTX2 and visual system homeobox 1 (VSX1). Studies of otx2 and vsx1 in *X. laevis* showed that their mRNAs were present early in retinal development but were not translated until late in retinal development, with the timing of translation dictated by their 3′ untranslated region (UTR)[75,76]. Reduction in the levels of dicer1, a protein that is required for miRNA processing, in *X. laevis*, led to a delay in the appearance of late-born cell types[74]. In addition, treatment of early retinae with cyclopamine, which blocks sonic hedgehog (shh) signalling, induced the translation of *otx2* and *vsx1* (REF. 76). A screen for miRNAs whose expression levels mimicked these effects — that is, they were present at high levels early in development and at low levels at later stages, and were reduced by treatment with cyclopamine — led to the identification of four miRNAs: miR-129, miR-155, miR-214 and miR-222. The sequences of these miRNAs suggested that they might target the 3′ UTRs of otx2 and vsx1. Depletion of these miRNAs showed that they could indeed regulate the translation of *otx2* and *vsx1* as well as the number of bipolar cells. These effects were unlikely to be due to effects on the cell cycle or apoptosis, as these parameters did not change when the miRNAs were depleted.

During the course of retinal development, there is normally an increase in the length of the cell cycle, which reflects increases in the length of all phases of the cell cycle[102]. Longer G1 and G2 phases also occur when cyclopamine is applied[103]. Thus, it has been proposed that the longer cell cycle in late RPCs (or after cyclopamine treatment) allows for a reduction in the concentration of the miRNAs that regulate the translation of *otx2* and *vsx1* (REF. 76). Their reduced concentration then enables *otx2* and *vsx1* translation and bipolar cell induction. miRNAs are thereby proposed to provide a link between the length of the cell cycle and the timing of retinal cell birth dates[76]. The development of the *X. laevis* retina occurs quite rapidly relative to that of mice and rats[5,8,12]. *X. laevis* may use mechanisms that are quicker to enact than those used in slower systems. For example, the bHLH gene *neurod1* has been shown to be regulated by a post-translation event, phosphorylation, in *X. laevis*[104] rather than by transcription, as it is in mice[105]. Therefore, it was of some interest to determine whether the temporal order of cell birth dates in mice might be regulated through the same mechanism as in *X. laevis* — that is, by miRNAs.

Removal of a conditional allele of *Dicer* in mice by Cre was carried out at the beginning of retinal development[77,106,107]. This resulted in a reduction in the number of cells with late fates and an increase in the number of cells with early fates. This was, in part, due to an expansion in the time period for the genesis of RGCs, one of the early fates[77,106]. There was also an increase in cell death as well as perturbations in the Notch and SHH pathways[77,106,107]. The effect on cell fates was the opposite to that observed in *X. laevis*. In *X. laevis*, miRNAs prevent the shift from early cell fates to late cell fates, whereas in mice miRNAs are required for this shift. In keeping with this difference, analysis of miRNAs in the mouse retina has not led to the discovery of any miRNAs that target *Otx2* or *Vsx1* (REF. 78) nor has the translation of these mRNAs been shown to be regulated in a temporal manner. However, several miRNAs, including let-7, miR-125 and miR-9, have been shown to be regulated by Dicer in mice, and functional studies of these miRNAs showed that they are at least in part responsible for the Dicer loss-of-function phenotype. Moreover, it was found that overexpression of these miRNAs was able to accelerate the early to late cell fate switch. Two of their targets, protogenin and lin-28b, were found to be able to induce the production of RGCs by late RPCs. As *let-7* and *lin-28* are heterochronic genes that were first discovered in *C. elegans*[108], these miRNAs may have a conserved role in the timing of development.

It is likely that transcription factors also contribute to the temporal progression of cell fates. Ikaros is a vertebrate homologue of Hunchback, a transcription factor that is required for the specification of early fates in the *D. melanogaster* ventral nerve cord[109]. Gain- and loss-of-function analyses of Ikaros in the mouse retina have revealed effects on the number of cells produced with early fates compared with the number produced with late fates, although photoreceptor fates were not affected as they would have been expected to be on the basis of their birth dates[110].

Extrinsic factors may also contribute to the regulation of the temporal progression of retinal cell fates. Extrinsic cues would be excellent candidates to mediate feedforward or feedback regulation. However, extrinsic cues are very unlikely to act as feedforward regulators, as the depletion of early-born cell types, such as RGCs[111–113] or amacrine cells[114], does not impair the production of later-born cell types. Furthermore, in favour

of intrinsic properties governing the timing of the onset of retinal cell differentiation, RGC differentiation is independent of retinal cues[29] or pre-existing RGCs[115]. By contrast, there are good examples of extrinsic cues that play a part in feedback inhibition. RGCs can limit the production of additional RGCs[60,116], and amacrine cells can limit the production of additional amacrine cells[30], through the production of soluble factors. SHH has been implicated as a negative regulator of RGC production in the chick[117] and mouse[118], and genetic experiments in the mouse have shown that growth/differentiation factor 11 (GDF11) negatively regulates RGC production[119]. The effect of GDF11 is accompanied by temporal changes in markers of RPCs, and GDF11 may prolong the early competence window required for RGC production. It does not, however, globally affect early cell fates: there is no change in the number of horizontal cells and it is not clear whether there is an effect on cones. Finally, a mechanism that involves counting the number of cell cycles does not seem to regulate temporal progression, as mice with too few[120,121] or too many[122] cell cycles still produce both early and late cell types in approximately the correct ratios.

Conclusions and future studies

The wide variability in the size and composition of retinal clones raised questions concerning the similarities and differences among RPCs. As described above, these questions are now beginning to be answered. There are data that reveal that specified RPCs produce specific pairs of daughter cells in terminal divisions. This can include pairs of very specific subtypes of cells, such as cones that express L opsin, or pairs of very disparate cell types, such as a rod and an amacrine cell. However, there are also data that suggest that stochastic processes are involved, with the proliferation patterns observed *in vivo* in zebrafish particularly well predicted by a stochastic model. To integrate these observations, it will be crucial to determine the nature of the RPCs that are upstream of the terminally dividing and specified RPCs. Are the upstream RPCs also specific types of RPCs? This information would enable one to determine whether there are specific lineages that extend beyond one terminal division (FIG. 2b). If there are extended lineages of this sort, it might indicate that the retina uses a strategy similar to that of the *D. melanogaster* nervous system (FIG. 4). However, even if such lineages exist, they need not be exclusive. The retina might use multiple strategies, with some RPCs making multiple cell types using stochastic processes. It may even be the case

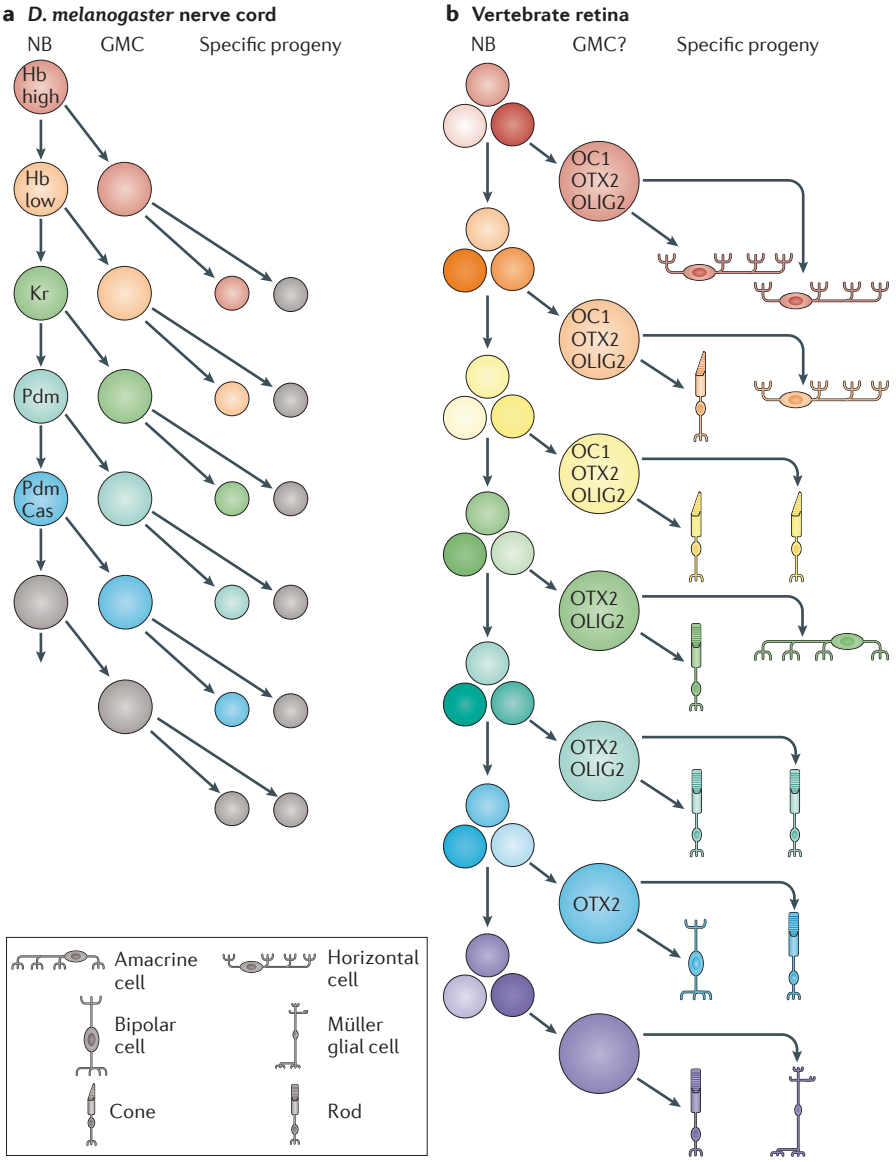

Figure 4 | **Potential parallels between the development of the vertebrate retina and that of the *Drosophila melanogaster* CNS. a** | Different areas of the *Drosophila melanogaster* nervous system develop using a lineage-based strategy in which neuroblasts (NBs) undergo asymmetrical divisions to produce NBs and ganglion mother cells (GMCs). The GMCs undergo terminal divisions to produce symmetrical or asymmetrical progeny. Each type of NB and GMC is specified by spatial and temporal factors, with the temporal identities of NBs derived from the expression of a series of transcription factors, here illustrated for the ventral nerve cord[109]. Asymmetrical fates among progeny neurons require the asymmetrical activity of Notch, presumably in the newly postmitotic daughter pairs[28]. **b** | The vertebrate retina may utilize some of the same strategies as *D. melanogaster*, using proliferative NBs and GMCs. The upper portion of the diagram shows NBs, the types of cells that give rise to medium to large clones when lineages are marked near the beginning of development. The different colours indicate changes in states of competence, with similar colours representing predictions of changes and overlaps in the expression of some of the same temporal transcription factors. These NBs produce terminally dividing retinal progenitor cells (RPCs), here illustrated as mouse oligodendrocyte transcription factor 2 (OLIG2)-expressing cells[51]. These terminally dividing RPCs share features of GMCs in that they undergo terminal divisions, are specified to produce different pairs of daughter cells at different times and their newly postmitotic progeny require Notch signalling to achieve a photoreceptor-plus-non-photoreceptor fate (not shown)[143]. Some additional proteins expressed in the terminally dividing cells are shown. Additional terminally dividing RPCs have been demonstrated (see FIG. 3 and TABLE 1 for examples) but are not shown here for clarity. The lineage relationships among NBs and terminally dividing RPCs have not been explored and will require additional studies. Cas, Castor; Hb, Hunchback; Kr, Kruppel; OC1, one cut domain family member 1; OTX2, orthodenticle homologue 2; Pdm, POU domain protein.

that both deterministic and stochastic mechanisms run in a single lineage, as suggested by the *Cdh6*-expressing RPCs, which are heavily biased to produce *Cdh6*-expressing RGCs, together with what may be a fairly stochastic subset of other retinal cell types. There may also be species-specific differences in the way that lineages are deployed, dictated by the speed of development and/or the demands of the species' lifestyle.

Another area to be explored is the role of miRNAs. How are they regulated? How do they regulate the birth dates of different cell types? How are they integrated with feedback signals and transcription factors that affect both temporal progression as well as individual fate choices? The power of current genetic and imaging methods, along with the availability of genomic-level information across many species, should make for an interesting next few years, as these questions are addressed.

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1. Dowling, J. E. *The Retina: An Approachable Part of the Brain* Revised edn (Belknap, 2012).
2. Gollisch, T. & Meister, M. Eye smarter than scientists believed: neural computations in circuits of the retina. *Neuron* **65**, 150–164 (2010).
3. Masland, R. H. The neuronal organization of the retina. *Neuron* **76**, 266–280 (2012).
4. Belecky-Adams, T., Cook, B. & Adler, R. Correlations between terminal mitosis and differentiated fate of retinal precursor cells *in vivo* and *in vitro*: analysis with the "window-labeling" technique. *Dev. Biol.* **178**, 304–315 (1996).
5. Rapaport, D. H., Wong, L. L., Wood, E. D., Yasumura, D. & LaVail, M. M. Timing and topography of cell genesis in the rat retina. *J. Comp. Neurol.* **474**, 304–324 (2004).
6. Wong, L. L. & Rapaport, D. H. Defining retinal progenitor cell competence in *Xenopus laevis* by clonal analysis. *Development* **136**, 1707–1715 (2009).
7. La Vail, M. M., Rapaport, D. H. & Rakic, P. Cytogenesis in the monkey retina. *J. Comp. Neurol.* **309**, 86–114 (1991).
8. Young, R. W. Cell differentiation in the retina of the mouse. *Anat. Rec.* **212**, 199–205 (1985).
9. Cherry, T. J., Trimarchi, J. M., Stadler, M. B. & Cepko, C. L. Development and diversification of retinal amacrine interneurons at single cell resolution. *Proc. Natl Acad. Sci. USA* **106**, 9495–9500 (2009).
10. Morrow, E. M., Chen, C. M. & Cepko, C. L. Temporal order of bipolar cell genesis in the neural retina. *Neural Dev.* **3**, 2 (2008).
11. Voinescu, P. E., Kay, J. N. & Sanes, J. R. Birthdays of retinal amacrine cell subtypes are systematically related to their molecular identity and soma position. *J. Comp. Neurol.* **517**, 737–750 (2009).
12. Holt, C. E., Bertsch, T. W., Ellis, H. M. & Harris, W. A. Cellular determination in the *Xenopus* retina is independent of lineage and birth date. *Neuron* **1**, 15–26 (1988).
13. Turner, D. L. & Cepko, C. L. A common progenitor for neurons and glia persists in rat retina late in development. *Nature* **328**, 131–136 (1987).
14. Wetts, R. & Fraser, S. E. Multipotent precursors can give rise to all major cell types of the frog retina. *Science* **239**, 1142–1145 (1988).
15. Fekete, D. M., Perez-Miguelsanz, J., Ryder, E. F. & Cepko, C. L. Clonal analysis in the chicken retina reveals tangential dispersion of clonally related cells. *Dev. Biol.* **166**, 666–682 (1994).
16. Reese, B. E., Necessary, B. D., Tam, P. P., Faulkner-Jones, B. & Tan, S. S. Clonal expansion and cell dispersion in the developing mouse retina. *Eur. J. Neurosci.* **11**, 2965–2978 (1999).
17. Turner, D. L., Snyder, E. Y. & Cepko, C. L. Lineage-independent determination of cell type in the embryonic mouse retina. *Neuron* **4**, 833–845 (1990).
18. Bassett, E. A. & Wallace, V. A. Cell fate determination in the vertebrate retina. *Trends Neurosci.* **35**, 565–573 (2012).
19. Hufnagel, R. B. & Brown, N. L. in *Patterning and Cell Type Specification in the Developing CNS and PNS: Comprehensive Developmental Neuroscience* Ch. 27 (eds Rubenstein, J. & Rakic, P.) 519–536 (Academic, 2013).
20. Johnston, R. J. Jr & Desplan, C. Stochastic mechanisms of cell fate specification that yield random or robust outcomes. *Annu. Rev. Cell Dev. Biol.* **26**, 689–719 (2010).
21. Rister, J., Desplan, C. & Vasiliauskas, D. Establishing and maintaining gene expression patterns: insights from sensory receptor patterning. *Development* **140**, 493–503 (2013).
22. Gomes, F. L. *et al.* Reconstruction of rat retinal progenitor cell lineages *in vitro* reveals a surprising degree of stochasticity in cell fate decisions. *Development* **138**, 227–235 (2011).
23. He, J. *et al.* How variable clones build an invariant retina. *Neuron* **75**, 786–798 (2012).
24. Cepko, C. L., Austin, C. P., Yang, X., Alexiades, M. & Ezzeddine, D. Cell fate determination in the vertebrate retina. *Proc. Natl Acad. Sci. USA* **93**, 589–595 (1996).
25. Jadhav, A. P., Cho, S. H. & Cepko, C. L. Notch activity permits retinal cells to progress through multiple progenitor states and acquire a stem cell property. *Proc. Natl Acad. Sci. USA* **103**, 18998–19003 (2006).
26. Jadhav, A. P., Mason, H. A. & Cepko, C. L. Notch 1 inhibits photoreceptor production in the developing mammalian retina. *Development* **133**, 913–923 (2006).
27. Li, X., Chen, Z. & Desplan, C. Temporal patterning of neural progenitors in *Drosophila*. *Curr. Top. Dev. Biol.* **105**, 69–96 (2013).
28. Spana, E. P. & Doe, C. Q. Numb antagonizes Notch signaling to specify sibling neuron cell fates. *Neuron* **17**, 21–26 (1996).
29. Kay, J. N., Link, B. A. & Baier, H. Staggered cell-intrinsic timing of ath5 expression underlies the wave of ganglion cell neurogenesis in the zebrafish retina. *Development* **132**, 2573–2585 (2005).
30. Belliveau, M. J. & Cepko, C. L. Extrinsic and intrinsic factors control the genesis of amacrine and cone cells in the rat retina. *Development* **126**, 555–566 (1999).
31. Belliveau, M. J., Young, T. L. & Cepko, C. L. Late retinal progenitor cells show intrinsic limitations in the production of cell types and the kinetics of opsin synthesis. *J. Neurosci.* **20**, 2247–2254 (2000).
32. Austin, C. P., Feldman, D. E., Ida, J. A. Jr & Cepko, C. L. Vertebrate retinal ganglion cells are selected from competent progenitors by the action of Notch. *Development* **121**, 3637–3650 (1995).
33. Cayouette, M., Barres, B. A. & Raff, M. Importance of intrinsic mechanisms in cell fate decisions in the developing rat retina. *Neuron* **40**, 897–904 (2003).
34. Reh, T. A. & Kljavin, I. J. Age of differentiation determines rat retinal germinal cell phenotype: induction of differentiation by dissociation. *J. Neurosci.* **9**, 4179–4189 (1989).
35. Watanabe, T. & Raff, M. C. Diffusible rod-promoting signals in the developing rat retina. *Development* **114**, 899–906 (1992).
36. Morrow, E. M., Belliveau, M. J. & Cepko, C. L. Two phases of rod photoreceptor differentiation during rat retinal development. *J. Neurosci.* **18**, 3738–3748 (1998).
37. Blackshaw, S. *et al.* Genomic analysis of mouse retinal development. *PLoS Biol.* **2**, E247 (2004).
38. Livesey, F. J., Young, T. L. & Cepko, C. L. An analysis of the gene expression program of mammalian neural progenitor cells. *Proc. Natl Acad. Sci. USA* **101**, 1374–1379 (2004).
39. Kim, D. S. *et al.* Identification of molecular markers of bipolar cells in the murine retina. *J. Comp. Neurol.* **507**, 1795–1810 (2008).
40. Trimarchi, J. M. *et al.* Molecular heterogeneity of developing retinal ganglion and amacrine cells revealed through single cell gene expression profiling. *J. Comp. Neurol.* **502**, 1047–1065 (2007).
41. Trimarchi, J. M., Stadler, M. B. & Cepko, C. L. Individual retinal progenitor cells display extensive heterogeneity of gene expression. *PloS ONE* **3**, e1588 (2008).
42. Roesch, K. *et al.* The transcriptome of retinal Muller glial cells. *J. Comp. Neurol.* **509**, 225–238 (2008).
43. Velculescu, V. E., Zhang, L., Vogelstein, B. & Kinzler, K. W. Serial analysis of gene expression. *Science* **270**, 484–487 (1995).
44. Brzezinski, J. A., Prasov, L. & Glaser, T. Math5 defines the ganglion cell competence state in a subpopulation of retinal progenitor cells exiting the cell cycle. *Dev. Biol.* **365**, 395–413 (2012).
45. Yang, Z., Ding, K., Pan, L., Deng, M. & Gan, L. Math5 determines the competence state of retinal ganglion cell progenitors. *Dev. Biol.* **264**, 240–254 (2003).
46. Feng, L. *et al.* MATH5 controls the acquisition of multiple retinal cell fates. *Mol. Brain* **3**, 36 (2010).
47. Brzezinski, J. A., Kim, E. J., Johnson, J. E. & Reh, T. A. Ascl1 expression defines a subpopulation of lineage-restricted progenitors in the mammalian retina. *Development* **138**, 3519–3531 (2011).
48. Holland, E. C. & Varmus, H. E. Basic fibroblast growth factor induces cell migration and proliferation after glia-specific gene transfer in mice. *Proc. Natl Acad. Sci. USA* **95**, 1218–1223 (1998).
49. Beier, K. T., Samson, M. E., Matsuda, T. & Cepko, C. L. Conditional expression of the TVA receptor allows clonal analysis of descendents from Cre-expressing progenitor cells. *Dev. Biol.* **353**, 309–320 (2011).
50. Roe, T., Reynolds, T. C., Yu, G. & Brown, P. O. Integration of murine leukemia virus DNA depends on mitosis. *EMBO J.* **12**, 2099–2108 (1993).
51. Hafler, B. P. *et al.* Transcription factor Olig2 defines subpopulations of retinal progenitor cells biased toward specific cell fates. *Proc. Natl Acad. Sci. USA* **109**, 7882–7887 (2012).
52. Nakamura, K., Harada, C., Namekata, K. & Harada, T. Expression of olig2 in retinal progenitor cells. *Neuroreport* **17**, 345–349 (2006).
53. Schuller, U. *et al.* Acquisition of granule neuron precursor identity is a critical determinant of progenitor cell competence to form Shh-induced medulloblastoma. *Cancer Cell* **14**, 123–134 (2008).
54. Godinho, L. *et al.* Nonapical symmetric divisions underlie horizontal cell layer formation in the developing retina *in vivo*. *Neuron* **56**, 597–603 (2007).
55. Suzuki, S. C. *et al.* Cone photoreceptor types in zebrafish are generated by symmetric terminal divisions of dedicated precursors. *Proc. Natl Acad. Sci. USA* **110**, 15109–15114 (2013).
56. Smallwood, P. M., Wang, Y. & Nathans, J. Role of a locus control region in the mutually exclusive expression of human red and green cone pigment genes. *Proc. Natl Acad. Sci. USA* **99**, 1008–1011 (2002).
57. Wang, Y. *et al.* Mutually exclusive expression of human red and green visual pigment-reporter transgenes occurs at high frequency in murine cone photoreceptors. *Proc. Natl Acad. Sci. USA* **96**, 5251–5256 (1999).
58. Roorda, A., Metha, A. B., Lennie, P. & Williams, D. R. Packing arrangement of the three cone classes in primate retina. *Vision Res.* **41**, 1291–1306 (2001).
59. Wernet, M. F. *et al.* Stochastic spineless expression creates the retinal mosaic for colour vision. *Nature* **440**, 174–180 (2006).
60. Poggi, L., Vitorino, M., Masai, I. & Harris, W. A. Influences on neural lineage and mode of division in the zebrafish retina *in vivo*. *J. Cell Biol.* **171**, 991–999 (2005).
61. Rompani, S. B. & Cepko, C. L. Retinal progenitor cells can produce restricted subsets of horizontal cells. *Proc. Natl Acad. Sci. USA* **105**, 192–197 (2008).
62. Genis-Galvez, J. M., Garcia-Lomas, V., Prada, F. & Armengol, J. A. Developmental study of axon formation in the horizontal neurons of the retina of the chick embryo. *Anat. Embryol.* **161**, 319–327 (1981).
63. Tanabe, K. *et al.* Cadherin is required for dendritic morphogenesis and synaptic terminal organization of retinal horizontal cells. *Development* **133**, 4085–4096 (2006).

64. Emerson, M. M., Surzenko, N., Goetz, J. J., Trimarchi, J. & Cepko, C. L. Otx2 and Onecut1 promote the fates of cone photoreceptor and horizontal cells and repress rod photoreceptors. *Dev. Cell.* **26**, 59–72 (2013).
65. Jones, I., Ng, L., Liu, H. & Forrest, D. An intron control region differentially regulates expression of thyroid hormone receptor β2 in the cochlea, pituitary, and cone photoreceptors. *Mol. Endocrinol.* **21**, 1108–1119 (2007).
66. Kay, J. N. *et al.* Retinal ganglion cells with distinct directional preferences differ in molecular identity, structure, and central projections. *J. Neurosci.* **31**, 7753–7762 (2011).
67. De la Huerta, I., Kim, I. J., Voinescu, P. E. & Sanes, J. R. Direction-selective retinal ganglion cells arise from molecularly specified multipotential progenitors. *Proc. Natl Acad. Sci. USA* **109**, 17663–17668 (2012).
68. Sidman, R. L. in *The Structure of the Eye* (ed. Smelser, G.) 487–506 (Academic, 1961).
69. Sinitsina, V. F. [DNA synthesis and cell population kinetics in embryonal histogenesis of the retina in mice]. *Arkhiv. Anatomii. Gistol. Embriol.* **61**, 58–67 (in Russian) (1971).
70. Hayashi, S. & McMahon, A. P. Efficient recombination in diverse tissues by a tamoxifen-inducible form of Cre: a tool for temporally regulated gene activation/inactivation in the mouse. *Dev. Biol.* **244**, 305–318 (2002).
71. Huang, S. & Moody, S. A. Asymmetrical blastomere origin and spatial domains of dopamine and neuropeptide Y amacrine subtypes in *Xenopus* tadpole retina. *J. Comp. Neurol.* **360**, 442–453 (1995).
72. Huang, S. & Moody, S. A. Three types of serotonin-containing amacrine cells in tadpole retina have distinct clonal origins. *J. Comp. Neurol.* **387**, 42–52 (1997).
73. Cochella, L. & Hobert, O. Embryonic priming of a miRNA locus predetermines postmitotic neuronal left/right asymmetry in *C. elegans*. *Cell* **151**, 1229–1242 (2012).
74. Decembrini, S., Andreazzoli, M., Barsacchi, G. & Cremisi, F. Dicer inactivation causes heterochronic retinogenesis in *Xenopus laevis*. *Int. J. Dev. Biol.* **52**, 1099–1103 (2008).
75. Decembrini, S., Andreazzoli, M., Vignali, R., Barsacchi, G. & Cremisi, F. Timing the generation of distinct retinal cells by homeobox proteins. *PLoS Biol.* **4**, e272 (2006).
76. Decembrini, S. *et al.* MicroRNAs couple cell fate and developmental timing in retina. *Proc. Natl Acad. Sci. USA* **106**, 21179–21184 (2009).
77. Georgi, S. A. & Reh, T. A. Dicer is required for the transition from early to late progenitor state in the developing mouse retina. *J. Neurosci.* **30**, 4048–4061 (2010).
78. La Torre, A., Georgi, S. & Reh, T. A. Conserved microRNA pathway regulates developmental timing of retinal neurogenesis. *Proc. Natl Acad. Sci. USA* **110**, E2362–E2370 (2013).
79. Salie, R., Niederkofler, V. & Arber, S. Patterning molecules: multitasking in the nervous system. *Neuron* **45**, 189–192 (2005).
80. Powell, L. M. & Jarman, A. P. Context dependence of proneural bHLH proteins. *Curr. Opin. Genet. Dev.* **18**, 411–417 (2008).
81. Hatakeyama, J., Tomita, K., Inoue, T. & Kageyama, R. Roles of homeobox and bHLH genes in specification of a retinal cell type. *Development* **128**, 1313–1322 (2001).
82. Novitch, B. G., Chen, A. I. & Jessell, T. M. Coordinate regulation of motor neuron subtype identity and pan-neuronal properties by the bHLH repressor Olig2. *Neuron* **31**, 773–789 (2001).
83. Akagi, T. *et al.* Requirement of multiple basic helix-loop-helix genes for retinal neuronal subtype specification. *J. Biol. Chem.* **279**, 28492–28498 (2004).
84. Hatakeyama, J. & Kageyama, R. Retinal cell fate determination and bHLH factors. *Semin. Cell Dev. Biol.* **15**, 83–89 (2004).
85. Hutcheson, D. A. *et al.* bHLH-dependent and -independent modes of *Ath5* gene regulation during retinal development. *Development* **132**, 829–839 (2005).
86. Mao, C. A. *et al.* Reprogramming amacrine and photoreceptor progenitors into retinal ganglion cells by replacing Neurod1 with Atoh7. *Development* **140**, 541–551 (2013).
87. Osterhout, J. A. *et al.* Cadherin-6 mediates axon-target matching in a non-image-forming visual circuit. *Neuron* **71**, 632–639 (2011).
88. Liu, Q. *et al.* Cadherin-6 function in zebrafish retinal development. *Dev. Neurobiol.* **68**, 1107–1122 (2008).
89. Ng, L. *et al.* A thyroid hormone receptor that is required for the development of green cone photoreceptors. *Nature Genet.* **27**, 94–98 (2001).
90. Applebury, M. L. *et al.* The murine cone photoreceptor: a single cone type expresses both S and M opsins with retinal spatial patterning. *Neuron* **27**, 513–523 (2000).
91. Onishi, A., Peng, G. H., Chen, S. & Blackshaw, S. Pias3-dependent SUMOylation controls mammalian cone photoreceptor differentiation. *Nature Neurosci.* **13**, 1059–1065 (2010).
92. Yanagi, Y., Takezawa, S. & Kato, S. Distinct functions of photoreceptor cell-specific nuclear receptor, thyroid hormone receptor β2 and CRX in one photoreceptor development. *Invest. Ophthalmol. Vis. Sci.* **43**, 3489–3494 (2002).
93. Hennig, A. K., Peng, G. H. & Chen, S. Regulation of photoreceptor gene expression by Crx-associated transcription factor network. *Brain Res.* **1192**, 114–133 (2008).
94. Koike, C. *et al.* Functional roles of Otx2 transcription factor in postnatal mouse retinal development. *Mol. Cell. Biol.* **27**, 8318–8329 (2007).
95. Nishida, A. *et al.* Otx2 homeobox gene controls retinal photoreceptor cell fate and pineal gland development. *Nature Neurosci.* **6**, 1255–1263 (2003).
96. Kechad, A. *et al.* Numb is required for the production of terminal asymmetric cell divisions in the developing mouse retina. *J. Neurosci.* **32**, 17197–17210 (2012).
97. Dasen, J. S. & Jessell, T. M. Hox networks and the origins of motor neuron diversity. *Curr. Top. Dev. Biol.* **88**, 169–200 (2009).
98. Jukam, D. & Desplan, C. Binary fate decisions in differentiating neurons. *Curr. Opin. Neurobiol.* **20**, 6–13 (2010).
99. Xiong, F. *et al.* Specified neural progenitors sort to form sharp domains after noisy Shh signaling. *Cell* **153**, 550–561 (2013).
100. Dyer, M. A. *et al.* Developmental sources of conservation and variation in the evolution of the primate eye. *Proc. Natl Acad. Sci. USA* **106**, 8963–8968 (2009).
101. Jacob, J., Maurange, C. & Gould, A. P. Temporal control of neuronal diversity: common regulatory principles in insects and vertebrates? *Development* **135**, 3481–3489 (2008).
102. Alexiades, M. R. & Cepko, C. Quantitative analysis of proliferation and cell cycle length during development of the rat retina. *Dev. Dyn.* **205**, 293–307 (1996).
103. Locker, M. *et al.* Hedgehog signaling and the retina: insights into the mechanisms controlling the proliferative properties of neural precursors. *Genes Dev.* **20**, 3036–3048 (2006).
104. Moore, K. B., Schneider, M. L. & Vetter, M. L. Posttranslational mechanisms control the timing of bHLH function and regulate retinal cell fate. *Neuron* **34**, 183–195 (2002).
105. Morrow, E. M., Furukawa, T., Lee, J. E. & Cepko, C. L. NeuroD regulates multiple functions in the developing neural retina in rodent. *Development* **126**, 23–36 (1999).
106. Davis, N., Mor, E. & Ashery-Padan, R. Roles for Dicer1 in the patterning and differentiation of the optic cup neuroepithelium. *Development* **138**, 127–138 (2011).
107. Iida, A., Shinoe, T., Baba, Y., Mano, H. & Watanabe, S. Dicer plays essential roles for retinal development by regulation of survival and differentiation. *Invest. Ophthalmol. Vis. Sci.* **52**, 3008–3017 (2011).
108. Ambros, V. MicroRNAs and developmental timing. *Curr. Opin. Genet. Dev.* **21**, 511–517 (2011).
109. Isshiki, T., Pearson, B., Holbrook, S. & Doe, C. Q. *Drosophila* neuroblasts sequentially express transcription factors which specify the temporal identity of their neuronal progeny. *Cell* **106**, 511–521 (2001).
110. Elliott, J., Jolicoeur, C., Ramamurthy, V. & Cayouette, M. Ikaros confers early temporal competence to mouse retinal progenitor cells. *Neuron* **60**, 26–39 (2008).
111. Brown, N. L., Patel, S., Brzezinski, J. & Glaser, T. Math5 is required for retinal ganglion cell and optic nerve formation. *Development* **128**, 2497–2508 (2001).
112. Wang, S. W. *et al.* Requirement for *math5* in the development of retinal ganglion cells. *Genes Dev.* **15**, 24–29 (2001).
113. Kay, J. N., Finger-Baier, K. C., Roeser, T., Staub, W. & Baier, H. Retinal ganglion cell genesis requires *lakritz*, a zebrafish *atonal* homolog. *Neuron* **30**, 725–736 (2001).
114. Li, S. *et al.* Foxn4 controls the genesis of amacrine and horizontal cells by retinal progenitors. *Neuron* **43**, 795–807 (2004).
115. McCabe, K. L., Gunther, E. C. & Reh, T. A. The development of the pattern of retinal ganglion cells in the chick retina: mechanisms that control differentiation. *Development* **126**, 5713–5724 (1999).
116. Waid, D. K. & McLoon, S. C. Ganglion cells influence the fate of dividing retinal cells in culture. *Development* **125**, 1059–1066 (1998).
117. Zhang, X. M. & Yang, X. J. Regulation of retinal ganglion cell production by Sonic hedgehog. *Development* **128**, 943–957 (2001).
118. Wang, Y., Dakubo, G. D., Thurig, S., Mazerolle, C. J. & Wallace, V. A. Retinal ganglion cell-derived sonic hedgehog locally controls proliferation and the timing of RGC development in the embryonic mouse retina. *Development* **132**, 5103–5113 (2005).
119. Kim, J. *et al.* GDF11 controls the timing of progenitor cell competence in developing retina. *Science* **308**, 1927–1930 (2005).
120. Ma, C., Papermaster, D. & Cepko, C. L. A unique pattern of photoreceptor degeneration in cyclin D1 mutant mice. *Proc. Natl Acad. Sci. USA* **95**, 9938–9943 (1998).
121. Das, G., Choi, Y., Sicinski, P. & Levine, E. M. Cyclin D1 fine-tunes the neurogenic output of embryonic retinal progenitor cells. *Neural Dev.* **4**, 15 (2009).
122. Dyer, M. A. & Cepko, C. L. Control of Muller glial cell proliferation and activation following retinal injury. *Nature Neurosci.* **3**, 873–880 (2000).
123. Lamb, T. D. Evolution of phototransduction, vertebrate photoreceptors and retina. *Prog. Retin. Eye Res.* **36**, 52–119 (2013).
124. Lamb, T. D., Collin, S. P. & Pugh, E. N. Jr. Evolution of the vertebrate eye: opsins, photoreceptors, retina and eye cup. *Nature Rev. Neurosci.* **8**, 960–976 (2007).
125. Lucas, R. J. Mammalian inner retinal photoreception. *Curr. Biol.* **23**, R125–R133 (2013).
126. Johnson, P. T., Williams, R. R., Cusato, K. & Reese, B. E. Rods and cones project to the inner plexiform layer during development. *J. Comp. Neurol.* **414**, 1–12 (1999).
127. Edqvist, P. H. & Hallbook, F. Newborn horizontal cells migrate bi-directionally across the neuroepithelium during retinal development. *Development* **131**, 1343–1351 (2004).
128. Liu, W., Wang, J. H. & Xiang, M. Specific expression of the LIM/homeodomain protein Lim-1 in horizontal cells during retinogenesis. *Dev. Dyn.* **217**, 320–325 (2000).
129. Poche, R. A. *et al.* Lim1 is essential for the correct laminar positioning of retinal horizontal cells. *J. Neurosci.* **27**, 14099–14107 (2007).
130. Arendt, D. Evolution of eyes and photoreceptor cell types. *Int. J. Dev. Biol.* **47**, 563–571 (2003).
131. Borst, A. & Euler, T. Seeing things in motion: models, circuits, and mechanisms. *Neuron* **71**, 974–994 (2011).
132. Demb, J. B. Cellular mechanisms for direction selectivity in the retina. *Neuron* **55**, 179–186 (2007).
133. Terakita, A. The opsins. *Genome Biol.* **6**, 213 (2005).
134. Wassle, H. Parallel processing in the mammalian retina. *Nature Rev. Neurosci.* **5**, 747–757 (2004).
135. Muranishi, Y. *et al.* An essential role for RAX homeoprotein and NOTCH-HES signaling in Otx2 expression in embryonic retinal photoreceptor cell fate determination. *J. Neurosci.* **31**, 16792–16807 (2011).
136. Marquardt, T. *et al.* Pax6 is required for the multipotent state of retinal progenitor cells. *Cell* **105**, 43–55 (2001).
137. Gazave, E. *et al.* Origin and evolution of the Notch signalling pathway: an overview from eukaryotic genomes. *BMC Evol. Biol.* **9**, 249 (2009).
138. Yaron, O., Farhy, C., Marquardt, T., Applebury, M. & Ashery-Padan, R. Notch1 functions to suppress cone-photoreceptor fate specification in the developing mouse retina. *Development* **133**, 1367–1378 (2006).

139. Simionato, E. *et al.* Origin and diversification of the basic helix-loop-helix gene family in metazoans: insights from comparative genomics. *BMC Evol. Biol.* **7**, 33 (2007).
140. Bringmann, A. *et al.* Muller cells in the healthy and diseased retina. *Prog. Retin. Eye Res.* **25**, 397–424 (2006).
141. Jadhav, A. P., Roesch, K. & Cepko, C. L. Development and neurogenic potential of Muller glial cells in the vertebrate retina. *Prog. Retin. Eye Res.* **28**, 249–262 (2009).
142. Bernardos, R. L., Barthel, L. K., Meyers, J. R. & Raymond, P. A. Late-stage neuronal progenitors in the retina are radial Muller glia that function as retinal stem cells. *J. Neurosci.* **27**, 7028–7040 (2007).
143. Mizeracka, K., Demaso, C. R. & Cepko, C. L. Notch1 is required in newly postmitotic cells to inhibit the rod photoreceptor fate. *Development* **140**, 3188–3197 (2013).

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