

VERTEBRATE NEURAL CELL-FATE DETERMINATION: LESSONS FROM THE RETINA

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Postmitotic neurons are produced from a pool of cycling progenitors in an orderly fashion during development. Studies of cell-fate determination in the vertebrate retina have uncovered several fundamental principles by which this is achieved. Most notably, a model for vertebrate cell-fate determination has been proposed that combines findings on the relative roles of extrinsic and intrinsic regulators in controlling cell-fate choices. At the heart of the model is the proposal that progenitors pass through intrinsically determined competence states, during which they are capable of giving rise to a limited subset of cell types under the influence of extrinsic signals.

CELL FATE

The cell type that a cell will become. This term does not imply commitment or differentiation, only that the cell will eventually become a certain type.

MÜLLER GLIA

The only retinal glia cell that derives from retinal progenitor cells.

PROGENITOR

A mitotic cell that is not capable of indefinite self-renewal and which will produce a limited repertoire of cell types.

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One key element in the construction of functional neural circuits during development is the generation of the correct types of neurons in the appropriate parts of the nervous system. It is now well established that this process of neural CELL-FATE determination is regulated by a combination of extrinsic and intrinsic influences¹⁻³. However, the relative extent to which these two influences contribute is not clear. For example, it is not known whether they are of equivalent importance and whether they are involved in regulating different aspects of cell-fate decisions. Recent studies of vertebrate neural cell-fate determination in several regions of the nervous system, including the retina, have clarified some of these issues.

During vertebrate retina development, six types of neuron and one type of glia (FIG. 1) are generated in an order that is generally conserved across all species studied: ganglion cells generated first, and rods, bipolar cells and MÜLLER GLIA produced last^{1,4-7} (FIG. 2). A body of classic studies has established several key features of this process. First, retinal PROGENITORS are MULTIPOTENT and subsets of progenitors are therefore not limited to the generation of only one or two cell types⁸⁻¹¹. Second, although there is a conserved order of genesis of the different cell types, many BIRTHDATING studies have indicated that there is considerable overlap in the times at which these cells are produced⁴⁻⁶. A recent study of early neurogenesis in zebrafish has shown that the process might be

more orderly than previously thought, with all ganglion cells within an area being generated before any cell type born later¹². However, as in all other vertebrates studied, there seems to be an overlap in the period of generation of later cell types.

An attractive hypothesis to accommodate these findings was that, once specified as retinal progenitors, the various cell fates of postmitotic neurons are determined by environmental signals^{9,10}. However, several experiments to test the influence of environmental signals on controlling cell fate have indicated that retinal progenitors are limited such that a particular progenitor can generate only a subset of cell types at a given time during development¹³⁻¹⁵. Environmental signals can alter the relative proportions of each cell type generated at a given time, but it cannot influence progenitors to make temporally inappropriate cell types. These and other findings led to the development of the COMPETENCE model of retinal development (FIG. 2), which proposed that progenitors pass through a series of competence states, during each of which the progenitors are competent to produce a subset of retinal cell types¹. Competence states seem to be intrinsically defined and thus cell fate choices are intrinsically regulated through the definition of progenitor competence. Within a given competence state, the generation of a particular type of cell is regulated by positive and negative extrinsic signals¹.

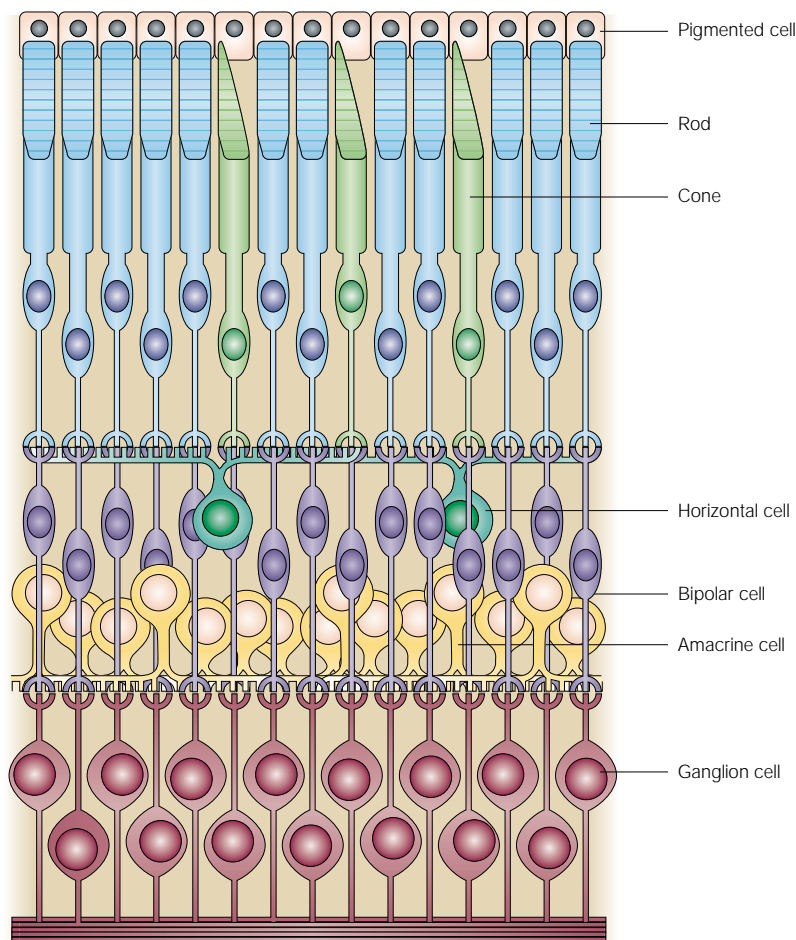


Figure 1 | **Histology of the adult mammalian retina.** The seven main cell types are shown, but it should be noted that in many species there are many distinct subtypes within each class of neuron⁸⁵. For example, there are at least 22 subtypes of amacrine cell in the rabbit retina⁸⁶.

MULTIPOTENT

The ability of a cell to take on more than one fate. Lineage analysis has defined retinal progenitor cells to be multipotent. By contrast, other experiments have shown that the cells are limited in their competence to make particular cell types at particular times. So competence is a temporally defined ability that does not show the overall potency of a cell; for example, early retinal progenitor cells cannot respond to late environments by producing late cell types within one to two days, even though their daughters will eventually become late cell types.

BIRTHDATE

The day a progenitor cell undergoes a terminal mitosis that results in production of a postmitotic cell.

As stated above, the competence model was originally formulated to account for findings on cell-fate determination in the vertebrate retina¹. However, it is now becoming clear that this is likely to describe a more general mechanism that underlies neural cell-fate determination in other regions of the vertebrate nervous system. Studies of cell-fate determination in the cerebral cortex, spinal cord and neural crest have highlighted important shared features and notable differences. In all cases, there is strong evidence for changes in progenitor competence over time, with progenitor competence being intrinsically defined^{16–20}. A similar mechanism might also be used in the invertebrate nervous system to generate distinct cell types in the developing *Drosophila melanogaster* eye²¹.

The competence model and its consequences **Progenitor differences over time.** A key aspect of the competence model is the finding that progenitors are intrinsically different in terms of their competence to produce distinct types of cells at different stages of development. Strong evidence for this came from *in vitro* HETEROCHRONIC transplant experiments in both

chick and rodents, in which progenitors from different stages of development were placed in an environment of a different age (either earlier or later). For example, early chick progenitors, which normally generate ganglion cells *in vivo*, generate ganglion cells regardless of the age of the environment that they are placed in¹³. Similar experiments in the rat indicated that mid-stage progenitors that normally produce amacrine cells and cone photoreceptors (along with some horizontal cells, rods and ganglion cells), and late progenitors that normally produce almost exclusively rod photoreceptors and a few bipolar neurons, do not change the type of progeny that they make when cultured in different environments^{14,15}.

These data lead to such questions as what defines the underlying cellular differences among progenitors at different times, how those differences define differing competences and how passage between one state and the next is regulated. Obvious mechanisms for control of these states are transcriptional programmes and/or protein expression, modification, accumulation or degradation. There is some evidence for transcriptional and translational differences among progenitors at different times of development. First, two markers discussed below that show heterogeneity in progenitors at a given time, **syntaxin-1a** and the VCL1.1 EPTOPE, also vary in their expression over time²². Second, progenitor responses to mitogens and the level of epidermal growth factor (EGF) receptor that is expressed on the progenitor cell surface change over time^{23,24}. Third, the expression of the cyclin kinase inhibitors (CKIs) p27 and p57 in mutually exclusive subsets of cycling progenitors^{25,26} raises possible links between control of cell-cycle exit and cell-fate determination. Last, it has been proposed that the level of another cyclin kinase inhibitor, p27^{Xic1}, increases over time in retinal progenitors in *Xenopus*²⁷. The accumulation of p27^{Xic1} above a certain threshold might drive the determination of Müller glia, normally the last cell type to differentiate in the retina in *Xenopus*, whereas overexpression of p27^{Xic1} in early progenitors can drive their progeny to the Müller glial fate at the expense of bipolar cells²⁷. In mice, however, the related and perhaps homologous CKI, p27^{Kip1} is dispensable for the generation of glial cells, although it regulates activation of glial cells²⁵. Mice deficient for p27 show inappropriate activation of glial cells, complete with all of the hallmarks of the typical pathological reaction²⁵.

Apart from these data, there is little information describing the intrinsic changes in progenitors over time, both at the RNA and protein levels. There is great potential for developing functional genomic technologies to supply key data for defining both the transcriptional programmes of neural progenitors, and changes in gene expression in progenitors during development. Such approaches should answer whether changes in competence are correlated with changing transcriptional programmes and also indicate transcriptional programmes that correlate with generation of different cell types, along with those associated with terminal DIFFERENTIATION²⁸.

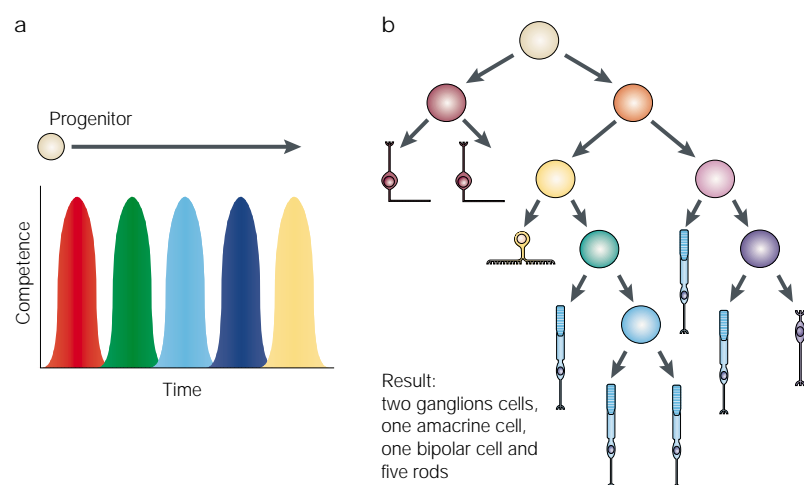


Figure 2 | The competence model of retinal cell-fate determination. a | A progenitor passes through waves of competence, indicated by different colours, during which it is competent to generate only a subset of types of postmitotic cells. A key feature of this model is that cells both acquire and lose the ability to make various cell types. This is in contrast to a model of progressive restriction, in which cells can make all cell types early in development, but then gradually lose the ability to make the early cells. **b** | The predicted lineage tree built up by cell divisions of multipotent progenitors over time. With each division, a progenitor generates two progeny, which might be either mitotic or postmitotic. The first division shown generates two progenitors, whereas all of the other divisions generate either a progenitor and a postmitotic cell or two postmitotic cells. Different colours of progenitor cells denote different competence states. Note that more than one type of progenitor is predicted to produce a particular cell fate, here a rod photoreceptor (blue). *In vivo* lineage analysis using retroviral labelling has shown that such multi-cell type clones are typical in the vertebrate retina^{8,9}.

Besides these molecular differences, there are several other characteristics of progenitors that change over time. First, their cell-cycle length increases throughout development, from a low of 14 hours to a peak of over 30 hours²⁹. Second, there is a marked shift over time in the types of cell divisions that progenitors undergo (FIG. 3). Early in development there is heavy production of mitotic cells within the developing retina, which far outstrips the production of postmitotic neurons. This production of progenitors can be accounted for only by large numbers of cells that divide to give rise to two cycling progenitors. Late in development the rate of production of progenitors from the progenitor pool plummets, with a net loss of mitotic cells and a large production of postmitotic neurons. In turn, these production figures can only be accounted for by dividing cells that give rise to two postmitotic neurons. So, large numbers of early progenitors divide symmetrically to produce progenitors, whereas late progenitors divide symmetrically to produce postmitotic neurons. It is likely that progenitors at early and mid-stages of development also undergo asymmetric divisions, generating one mitotic and one postmitotic daughter cell, as in the developing cortex^{30–32}.

Several lines of evidence indicate the existence of asymmetric divisions in the retina that produce mitotic and postmitotic progeny, particularly during the middle period of neuronal production. First, an analysis of a large cohort of relatively late clones generated in the rat, labelled using replication-incompetent retroviruses, shows that there is no bias towards even numbers in

the distribution of clone sizes⁹. Such a periodic distribution of clone sizes would be expected if all divisions are symmetric. Although cell death can obscure the original clone sizes, this is not much of a confounding issue at this time in the rat or mouse, as the death rate among the vast majority of late generated cells (rods) is estimated to be less than 5% (REFS 33,34). Second, the clone-size distribution among relatively early clones in the mouse indicates that production of two postmitotic cells during this period might be rare⁸. Although almost 35% of clones generated by infection at mouse embryonic day 14 (E14) were single-cell clones, fewer than 10% were clones that contained two to ten cells⁸. The single-cell clones were almost certainly the result of viral integration during M phase into a cell that became postmitotic immediately after infection, as most of the cell types in these clones were the cell types produced at this time (that is, cones, ganglion cells and amacrine cells). If two postmitotic cells were typically produced in a symmetric division during this period, then many two-cell clones would be seen when integration of the viral genome took place in a progenitor that went on to make two postmitotic cells in the next division. In fact, the frequency of such two-cell clones should be at least as high as that of one-cell clones, as in a model of retinal neurogenesis in which all divisions were symmetric, the frequency of symmetric postmitotic divisions would increase over time (FIG. 3). The fact that two-cell clones are very much less frequent than predicted suggests that both asymmetric and symmetric postmitotic divisions must occur.

Although alternative explanations for these observations can be made, together they argue that asymmetric divisions take place during the middle period of retinal neuron production. Division patterns and the order of genesis of retinal cell types have been studied in dissociated cells *in vitro*³⁵. However, many progenitors differentiate immediately under such conditions¹² and low numbers of cells continue to divide in such cultures³⁵. Therefore, direct observations of the *in vivo* behaviour of progenitors will be the most faithful way to determine these patterns.

The third aspect of progenitor behaviour that changes over time is the appearance of neural stem cell characteristics in late postnatal and adult life that are absent in the prenatal retina, operationally defined as the ability to generate neurosphere-like cultures from retinal tissue. It is likely that all of these temporal differences in progenitor behaviour are mediated to a considerable degree by intrinsic differences among progenitors of different ages, rather than changing extracellular environments. For example, mid- and late-stage progenitors showed differential sensitivity at different ages to the mitogenic action of fibroblast growth factors (FGFs) and EGF or transforming growth factor (TGF), with an increase in the levels of surface EGF receptor over time^{23,24}.

Does a motor drive changes in competence? Given that competence states seem to be intrinsically defined, a key question is how a progenitor moves between competence states. Of particular interest is whether there is

COMPETENCE

The ability of a cell to respond to a cue or set of cues to produce a defined outcome.

HETEROCHRONIC

A term usually used in the context of an experiment designed to test the role of the environment from one temporal window on cells from a different one, for example, transplantation of embryonic cells into a postnatal animal, or exposure of postnatal cells to embryonic cells in culture.

VC1.1 EPITOPE

An N-linked carbohydrate that is present in several glycoproteins and proteoglycans.

DIFFERENTIATION

The elaboration of particular characteristics expressed by an end-stage cell type, or by a cell *en route* to becoming an end-stage cell. This term is not synonymous with commitment, but differentiation features are used to determine when a cell is committed.

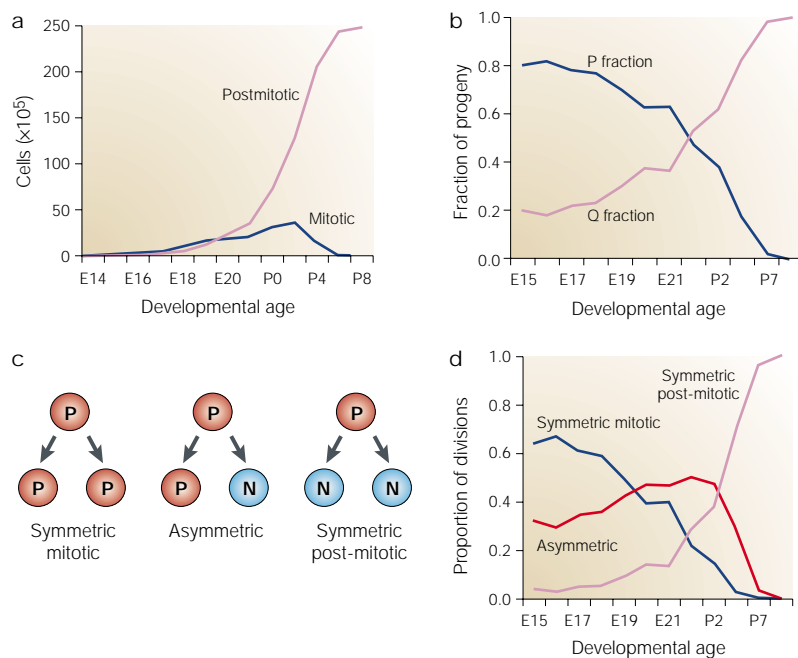


Figure 3 | Production of postmitotic cells in the neural retina over time. **a** | Numbers of neurons (postmitotic cells) and cycling progenitors in the rat retina over developmental time. (Data taken from REF. 29.) **b** | Proportions of the progeny of cell divisions exiting the cell cycle (Q, or quitting fraction) or continuing to proliferate (P fraction)⁹⁰ on each day of development in the rat retina. Calculated from data on absolute numbers of mitotic and postmitotic cells in the developing rat retina on each day of development in REF. 29. **c** | Modes of division possible for a progenitor. P indicates a mitotic progenitor cell and N indicates a postmitotic neuron. **d** | Predictions of the relative proportions of each of the three division types available to a progenitor in the developing rat retina, calculated from the P and Q fractions shown in **c**. All of the postmitotic cells produced in the retina can be accounted for by a simple mix of symmetric mitotic and postmitotic divisions, so that the relative proportions of each division type would be the same as the P and Q fractions. However, asymmetric divisions are a common feature of invertebrate and vertebrate neurogenesis⁹¹ and are likely to also occur in the vertebrate retina. One proposed solution to calculating the relative proportions of all three division types in the cerebral cortex is to calculate relative proportions using the binomial theorem⁹⁰, such that if $P + Q = 1$, then $P^2 + Q^2 + 2PQ = 1$, where P^2 are symmetric mitotic divisions, Q^2 symmetric postmitotic divisions and $2PQ$ asymmetric divisions. The relative proportions of each division type can therefore be calculated from the observed P and Q values. (On graph: E, embryonic day; P, postnatal day)

a need for an active signal, whether there is an internal motor to drive a progenitor between competence states, and whether the generation of committed progeny somehow alters the competence of progenitors. One possibility is that an environmental signal derived from postmitotic neurons might move progenitors between competence states. As discussed below, several types of retinal neuron produce signals that inhibit the generation of those neurons, thereby producing a negative-feedback system that regulates the proportions of the different cell types^{14,36,37}. Such a signal could act by driving progenitors from the competence state within which they can produce those cell types and into the next competence state in which they cannot provide more of the earlier born cell type¹⁴. Alternatively, such signals could act transiently to repress the production of the earlier cell types, or to promote the production of other cell types at the expense of the cells that generate the signals. This last possibility might not occur, as feedback inhibition of amacrine production can be

separated from feedback promotion of cone fates in the developing retina, arguing that separate signals are required to promote the genesis of cones and suppress the genesis of amacrine cells¹⁴.

Regardless of whether changes in competence states are driven by extrinsic signals or by an intrinsic motor within progenitors, it is not known what a shift in competence would mean in terms of cellular and molecular biology. Possibilities include modulating the activity or availability of a key cell surface receptor or signal-transduction pathway component, changes in transcription-factor expression or activity, or large-scale changes in gene expression. The question of how a progenitor moves between competence states is intimately related to the definition of competence states. So, changes in competence states are likely to become clearer once we understand more about the transcriptional and translational programmes of progenitors and how they differ between the observed competence states.

Progenitor heterogeneity. All of the elements of the competence model discussed could be modelled such that there is a single, homogenous population of progenitor cells at any given time point, which then synchronously passes from one competence state to another. However, it is likely that the population of progenitors at any one time is more complex, as there is evidence for progenitor heterogeneity at several points in development.

This laboratory found that a large proportion of progenitors at an early point in development express two markers that are indicative of some of the postmitotic neurons generated at that time²². Moreover, these two markers, VC1.1 and syntaxin-1a (markers of amacrine and horizontal cells), seem to be expressed by a subset of progenitors biased to produce those cell types at this time²². There is also more general evidence of progenitor heterogeneity. For example, two neurogenic basic HELIX-LOOP-HELIX (bHLH) transcription factors, **Mash1** (mammalian achaete scute homologue 1) and **Math5** (mammalian atonal homologue 5), are expressed only in subsets of progenitors^{38,39}. Finally, studies of the CIP/KIP family of CKIs discussed above have recently led to the definition of two classes of progenitor during the embryonic period; one set that expresses and relies on p27^{Kip1} for cell-cycle exit, and another set that expresses p57^{Kip2} and relies on it for prevention of re-entry into the cell cycle after exiting^{25,26,40}. Cells do not express both these genes and it seems that the subset of cells heading towards the amacrine pathway expresses p57^{Kip2} (REF. 26).

Therefore, a more complex model proposes that a heterogeneous pool of progenitors passes through competence states and different sub-populations are biased to give rise to different subsets of cell types. It might also be that a strict order of competence states is not followed by all progenitors, with some perhaps skipping certain states. If there are biased subsets of progenitors, it will be of interest to investigate whether there are lineal relationships between different biased subsets.

HELIX-LOOP-HELIX
A structural motif present in many transcription factors, which is characterized by two α -helices separated by a loop.

Table 1 | Genes expressed in both progenitors and postmitotic cells

Gene name/antigen	Protein class	Postmitotic expression
Genes broadly expressed in progenitors		
Notch ^{13,45–47,81,92,93}	Receptor	MG
Hes-1 (REFS 47,82)	TF	MG
Pax6 (REFS 55,83)	TF	AC, GC, HC
Rax ^{47,52}	TF	MG
Prox-1 (REFS 55,84)	TF	HC
Optx-2 (REFS 94,95)	TF	INL, GC
Chx-10 (REFS 55–57)	TF	BP
p27Xic1 (REF 27)	CKI	MG?
NeuroD ^{96–98}	TF	RPh, transiently AC
Genes expressed in subsets of progenitors		
p57Kip2 (REF 26)	CKI	AC (subset)
p27Kip1 (M. A. Dyer and C.L.C., submitted)	CKI	MG
Neurofilament ¹³	Cytoskeletal	GC
β3-nAChR ⁹⁹	Receptor	GC
Syntaxin-1a (REF 22)	Synaptic vesicle	AM, HC
VC1.1 (REF 22)	Unknown	AM, HC

(AC, amacrine cell; BP, bipolar neuron; CKI, cyclin kinase inhibitor; GC, ganglion cell; HC, horizontal cell; INL, inner nuclear layer; TF, transcription factor; MG, Müller glia; RPh, rod photoreceptor.)

Control of cell fate within a competence state
An extreme interpretation of the available data suggests that competence states are intrinsically determined at the level of gene and protein expression, whereas cell fate within a competence state might be regulated to a large degree by extrinsic signalling.

Extrinsic regulators of cell fate. Extrinsic signals can regulate retinal cell fate at two points. First, there are soluble factors produced by postmitotic neurons that provide feedback inhibition to progenitors to regulate cell-fate choices and which, at least for amacrine cells, seem to act on the progenitor before M phase¹⁴. Second, several factors have been shown to act on postmitotic cells to influence cell fate, including members of the ciliary neurotrophic factor (CNTF)/leukaemia inhibitory factor (LIF) family that can drive cells fated to be rods to express features of the bipolar neuron phenotype and fail to express rod markers⁴¹. In this case, although the cells are specified to become rods, an extrinsic signal can change the fate of these cells. The amino acid taurine also seems to act on the postmitotic precursor to promote rod differentiation⁴², although it could also be acting at the progenitor level. However, to sound a note of caution about drawing general conclusions from these observations, it is important to note that photoreceptors might be a special case in terms of postmitotic respecification. Many factors have been shown to influence positively and negatively postmitotic rod differentiation *in vitro*, including *sonic hedgehog*, retinoic acid and EGF⁴³, but there are at present no examples of postmitotic respecification for the other retinal cell types.

Notch signalling. No discussion of the intercellular signalling systems that could regulate neural cell fate would be complete without addressing the possible role

of **Delta-Notch** signalling. Originally discovered in *Drosophila* (for review, see REF 44), Notch signalling has been shown to be involved in the differentiation of both neurons and glia in the vertebrate retina and developing forebrain^{13,45–48}. However, it is not clear whether changes in Notch signalling are instructive or simply permissive in regulating cell-fate choices in vertebrates. Transient Notch signalling instructs neural crest progenitors to switch their competence from neurogenesis to gliogenesis⁴⁹ and data from T-cell development in the haematopoietic system indicates that Notch can actively regulate cell-fate choices⁵⁰. During the late stages of rat retinal development, introduction of Notch favours the development of Müller glia at the expense of neurons⁴⁷. This might be due to a block in the production of postmitotic cells from earlier, neuron-only competence states. Cells with high Notch signalling might pass into the last competence state, one in which they make Müller glia, without producing neurons *en route* to this state. Extensive production of Müller glia may then take place in the presence of Notch signalling, which might indicate a permissive rather than instructive role for Notch. In the early chick retina, reduced Notch signalling has been shown to induce production of ganglion cells¹³. This is distinct from the presumably more specific signal that mediates ganglion cell feedback inhibition³⁷.

A further issue is whether Notch signalling also regulates progenitor proliferation directly, as it seems to in the *Drosophila* wing disc⁵¹, or is simply permissive for proliferation⁴⁴. The data from introduction of activated Notch into retinal progenitors does not allow a conclusion to be made on this point. Introduction of the constitutively active intracellular domain of *Xenopus laevis* Notch into *Xenopus* early progenitor cells led to an arrest of both cell division and differentiation⁴⁵. By contrast, introduction of a similar allele of the mouse Notch gene into late rat retinal progenitor cells led to a stimulation of cell division and acquisition of Müller glial-like characteristics^{46,47}. The fact that different aged progenitors were transduced might account for this difference, but more work will need to be done to determine the role of Notch in retinal proliferation.

Intrinsic regulation of cell fate. In contrast with studies of extrinsic regulation of cell-fate choices, we know little about the intrinsic regulation of cell-fate choices. As discussed above, progenitor competence states are probably defined to some degree by gene expression, but we are just beginning to define the transcriptional programmes of retinal progenitors. Several transcription factors, receptors and signal-transduction pathway components that are expressed in retinal progenitors have been identified in recent years (TABLE 1). Interestingly, most of these transcription factors are also expressed in one or more types of postmitotic progeny. The significance of this pattern of expression is unclear in most cases. For example, the paired-type HOMEBOX genes, **Pax6** and **Rax**, have null alleles in mice that result in the lack of an eye^{52–54}, and later functions in differentiated neurons have not been addressed. However, in the case of the

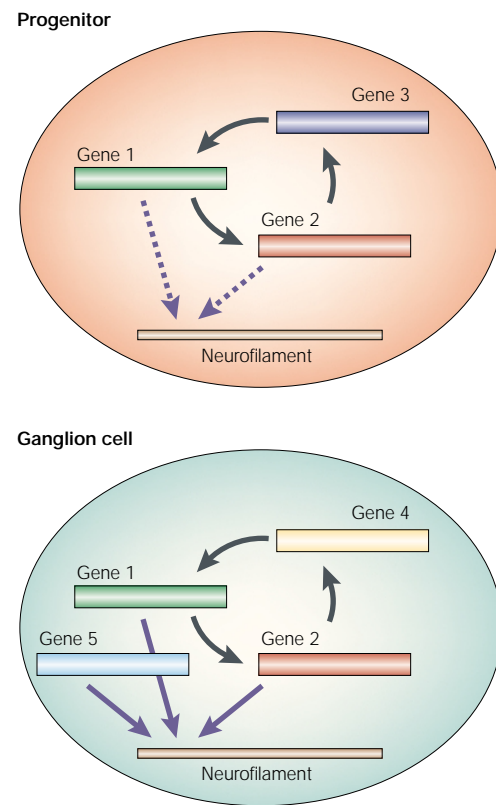
SPECIFICATION

A cell that is competent to make a particular cell type might begin to differentiate along the pathway to become that cell type, but it might not be committed to that fate, that is, its differentiation can be reversed and another fate can be achieved through respecification.

HOMEBOX

A sequence of about 180 base pairs that encodes a DNA-binding protein sequence known as the homeodomain.

Box 1 | Transcriptional networks in progenitors and progeny



Examples of theoretical transcription networks that might underlie competence and a committed state for ganglion cells are shown. In the progenitor that is competent to make a ganglion cell, a simple network among three genes, each encoding a transcription factor, is shown. One or more of these might be a factor, such as paired box gene 6 (Pax6) (REFS 71,72,81–83) or mammalian atonal homologue 5 (Math5) (REFS 36,84), which is also expressed in committed ganglion cells. This network is proposed to be unstable in the progenitor. In order for commitment and full differentiation to occur, we propose that the network needs to be stabilized. Stabilization might occur through lack of inhibition by Notch, and/or a decision to exit the cell cycle, as well as a lack of inhibition by a small molecule made by mature ganglion cells (feedback inhibition, REF 34) (also see BOX 2). One transient output of this network is transcription of a gene that has no function in the progenitor cell, here proposed to be neurofilament, which was found to be expressed in progenitor cells only during the stage when ganglion cells were being made¹³. Once commitment occurs, an overlapping set of transcription factors is proposed to set up a stable network that allows full differentiation. As shown, some of the same transcription factors present in the progenitor cell might persist in the committed cell (for example, Pax6 or Math5). In the committed state, neurofilament expression would be stabilized by expression of the transcription factors made by gene 1 and gene 2, as well as gene 5.

Caenorhabditis elegans Chx10 gene (ceh-10 homeo-domain-containing homologue), another paired-type homeobox gene expressed in retinal progenitor cells and bipolar neurons^{55–57}, a retina is made in the absence of its function⁵⁸. This retina is tenfold smaller than normal, but does differentiate to the point at which an assessment can be made of the effect that results from loss of Chx10 on bipolar cells. The result is clear: no bipolar cells are seen in this mutant⁵⁸. The retina therefore depends on Chx10 both for normal proliferation and bipolar development. It is not clear, however, whether expression in progenitors is required to make bipolar cells, or whether expression in differentiating cells is sufficient. Such a distinction will be important to understand the functions of these genes and their molecular mechanisms, including whether they have different target genes in progenitor cells and differentiated cells.

In addition to transcription factors, there are several other examples of genes expressed in progenitors that are characteristic of their postmitotic progeny, although those genes do not seem to have specific functions in the progenitors. This phenomenon, which can be viewed as precocious differentiation, is not restricted to the retina, as early expression of neurofilament by fore-brain progenitors has been well described^{59–61}. These data indicate that progenitors might already express, albeit at low levels, some of the genes required in their progeny, as is the case in haematopoietic stem cells⁶². In the retina, this precocious expression might indicate an

intrinsic bias in competence, as has been shown for one subset of progenitors²². This raises the interesting possibility that the transcriptional networks expressed in both progenitors and progeny have common and unique components. This would explain the expression in progenitors of genes that normally function only in differentiated cells and reflect a bias to produce certain cell types (BOX 1). The precociously expressed differentiation genes might prove useful as reporters or markers of particular competence states²².

Other data on intrinsic determinants in the retina address the later stages of phenotypic differentiation, rather than cell-fate choices. Several transcription factors that are expressed in specific postmitotic cell types, and that are necessary for the late phenotypic differentiation of those cells, have been identified, including the POU-domain transcription factor Brn3 family in ganglion cells and the cone-rod homeobox-containing gene (*Crx*) in photoreceptor cells^{63–69}. The transcriptional networks controlled by those late differentiation genes are beginning to be described²⁸.

Towards an integrated model

Cycling retinal progenitors make several decisions during the cell cycle regarding the fate of their progeny (BOX 2). One decision concerns the type of cell division that they will undergo: symmetric, in which both daughters are mitotic or postmitotic, or asymmetric, in which one daughter is mitotic and one is postmitotic (see above and

Box 2 | An integrated model of retinal cell-fate decision-making

An asymmetric division by a progenitor cell that is competent to make a ganglion cell is shown. The cell integrates information from the environment with the intrinsic competence information to determine whether to make a committed ganglion cell. If Notch or negative feedback signals are non-permissive, its daughters may continue to cycle and eventually pass into another competence state.

We postulate that the signals described are being assessed during the cell cycle, and make some estimates as to when these signals are being assessed. However, the precise timing of these events is at present unclear, except as noted below.

1 | Cycling cell makes a decision regarding the mitotic fate(s) of its daughters.

2 | Cycling cell assesses positive and negative environmental cues that will determine whether it can make the daughter cell type that it is competent to make (for example, ganglion cell feedback inhibition signal).

3 | Determinants concerning mitotic fate and cell fate are organized for transmission to the daughter cells.

4 | Synthesis of the cyclin kinase inhibitor p27 is initiated and then maintained in a daughter cell that inherits the decision to exit the cell cycle. The timing of this event has been determined to be just before or after M phase in rodent cells (M. A. Dyer and C.L.C., submitted).

5 | Notch signalling levels are assessed throughout the cell cycle. The signals might affect the decision to exit the cycle, to differentiate, to commit and, possibly, to influence some types of cell-fate choices. Reduced Notch signalling might lead to stabilized gene expression patterns through a mechanism similar to that used in the *Drosophila melanogaster* sensory organ precursor cell^{87–89}. In this cell, achaete-scute (ASC) levels rise, owing to a lack of negative basic helix-loop-helix (bHLH) activity induced by Notch, to confer stable expression of ASC by binding to a particular site in the ASC promoter^{87,88}.

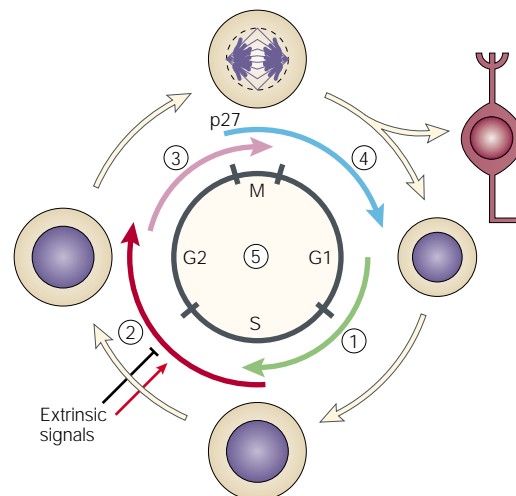


FIG. 3). In contrast with most models of the cell cycle in which a cell decides to progress into S phase when in G1, we propose that the decision for a cell to exit the cell cycle is made in the previous cycle by a progenitor cell. We further propose that the information regarding whether or not to continue cycling is distributed to the daughter cells during cytokinesis, in some cases asymmetrically. This proposal is based on two observations of gene expression in cells just as they exit M phase. Waid and McLoon found that RA4 protein, a marker of chick ganglion cells, was not expressed in S or G2 phase cells, but was first detectable in cells within 15 minutes of exiting M phase⁷⁰. Because transcription does not take place during M phase, it is most probably the case that RA4 mRNA is made during G2. Furthermore, because many divisions are asymmetric at this time in development, it is likely that only one daughter inherited the RA4 mRNA. This is in keeping with the idea that this daughter was designated to be the postmitotic daughter. Second, p27^{Kip1} protein expression was first detected either just before or just after M phase (M. A. Dyer and C.L.C., submitted). Because we believe that p27^{Kip1} marks the cells that are about to exit the cell cycle, the implication is again that G2 cells make the mRNA that is passed asymmetrically to the daughter designated to be postmitotic. Finally, COMMITMENT to the amacrine fate seems to occur in G2 (REF. 14), and again, because many divisions during this period of development are asymmetric, it is most likely that the daughter designated to be postmitotic inherits the amacrine decision. These observations on amacrine cells, ganglion cells and their

progenitors indicate that the decision to be mitotic or postmitotic, as well as the decision to take on a particular fate, might be made by the progenitor cell.

Progenitors might also make decisions regarding the fate of their mitotic progeny, assuming that there are several distinct subsets of progenitor types. These decisions are influenced by all of the information available to the progenitor, including its intrinsically determined competence state and extrinsic signals, in some cases in the form of feedback inhibition (amacrine and ganglion cells) and, in others, in the form of stimulators (photoreceptors). It is important to note, however, that mitotic progenitor cells might not make commitment decisions for all fates. Commitment to the rod photoreceptor fate seems to occur in G0 cells, for at least a subset of cells fated to be rods⁴¹.

Therefore, one challenge is to understand how all this information is interpreted and managed by progenitors to generate the different cell types. Is there a hierarchy of decision-making whereby cells decide: first, for example, that both daughter cells will exit the cell cycle; and second, to use the available extrinsic and intrinsic signalling information to decide on the fate of those cells? Or do cells make a single, integrated decision? The available data indicate that it is unlikely to be a single decision, given that differentiation induced by reduced Notch signalling is independent of feedback inhibition of cell-fate choice³⁷ and also that several factors can affect rod photoreceptor-fate choice after cell division. However, recent studies in the developing *Drosophila* eye have indicated that several distinct,

COMMITMENT

An irreversible decision to produce or become a particular cell type. This is defined operationally as the refusal of a cell to change its fate when exposed to various different environments.

general signals, including Notch, can be integrated in a single enhancer to regulate expression of genes that are associated with differentiation of specific cell types^{71–73}.

Beyond the retina

Studies of neural cell-fate determination in the cerebral cortex, spinal cord and neural crest have indicated that the competence model is likely to be a common mechanism for generating several cell types from a progenitor population over time. In all of these tissues, many of the key features of this model have been observed, including intrinsically defined competence states, changes in competence over time and the ability of extrinsic factors acting on progenitors to affect the cell-fate choices of their progeny.

Within the developing cerebral cortex, as in the retina, progenitors pass through phases during which they are competent to produce cells of a given laminar fate. Cortical progenitors also seem to pass through competence states during which they give rise first to neurons and then to glia, as also occurs in the neural crest^{49,74}. One important difference between retina and cortex is that early cortical progenitors seem to be able to jump ahead, in terms of their state of competence, when placed in a late environment^{16,19}. This ability to jump ahead might indicate that the cortex fits a model of progressive restriction, in which early progenitors can make all cell types, and then gradually lose this ability over time. This is in contrast to the behaviour of retinal progenitors, which do not seem to be able to make all cell types early. However, it should be noted that this ability of cortical progenitors was shown *in vivo*, whereas the lack of ability to jump ahead for retinal progenitors was shown *in vitro*. Many attempts to do *in vivo* transplantation of progenitors to the retina failed owing to problems with integration of transplanted cells into the very thin retinal neuroepithelium. Spinal cord progenitors are also multipotent and generate different cell types at different times^{17,75}.

A second common feature of cell-fate determination in these diverse parts of the nervous system is the ability of extrinsic factors to regulate cell-fate choices by acting during certain phases of the progenitor cell cycle. Cortical progenitors can be influenced in the cell-fate choices of their progeny by extrinsic signals in late S/early G2 phase in the cell cycle⁷⁶, and retinal progenitors can be negatively influenced in their fate choices up to at least M phase¹⁴. Similarly, ventral spinal cord progenitors adopt a motor neuron fate if exposed to sonic hedgehog until late S phase, and adopt an alternative interneuron fate in the absence of hedgehog¹⁸.

Feedback signalling from postmitotic cells to influence progenitor decisions also might be common to all of these three regions of the developing nervous system. In the spinal cord, postmitotic motor neurons provide feedback signals to the progenitor population that are required for the generation of a class of interneurons⁷⁷. As has been shown for two cell types in the retina, it has been suggested that cortical neurons might provide negative feedback to progenitors to repress the further production of those cell types^{15,19}. Last, reminiscent of the heterogeneity of retinal progenitors, spinal cord progenitors show a marked spatial organization, with distinct subsets that are specified to generate different types of neurons, defined by their expression of homeodomain transcription factors⁷⁸.

Finally, it has been suggested that a similar mechanism for regulating cell fate might operate in invertebrates, with the best example coming from studies of *Drosophila* eye development²¹. During this process, different postmitotic cells are generated by progenitors at different times under the influence of EGF receptor signalling⁷⁹. It has been proposed that this is achieved by progenitors changing over time²¹, such that the same extrinsic signal is interpreted differently, a situation analogous to vertebrate progenitors changing their competence over time.

Conclusions

Studies on the control of cell-fate determination in the vertebrate retina have provided an insight into a possible general mechanism whereby several cell types are generated in an orderly fashion over time from a progenitor population. This mechanism involves changes in progenitor competence over time, and the generation of specific postmitotic progeny from those progenitors by positive and negative extrinsic signals. As advances are made in understanding the cellular and molecular biology of progenitor competence, it will be interesting to see how general this mechanism is and whether it also applies to non-neuronal progenitor populations, such as haematopoietic stem cells⁸⁰, whose competence changes over the life of the organism.

Links

DATABASE LINKS [syntaxin-1a](#) | [p27^{Kip1}](#) | [Mash1](#) | [Math5](#) | [p57^{Kip2}](#) | [CNTF](#) | [LIF](#) | [sonic hedgehog](#) | [Delta](#) | [Notch](#) | [Pax6](#) | [Rax](#) | [Chx10](#) | [Brn3](#) | [Crx](#)

ENCYCLOPEDIA OF LIFE SCIENCES [Visual system development in vertebrates](#) | [Neural development: bHLH genes](#) | [Cell cycle: regulation by cyclins](#)

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