The mystery of intracellular developmental programmes and timers

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Abstract
There has been a revolution in understanding animal development in the last 25 years or so, but there is at least one area of development that has been relatively neglected and therefore remains largely mysterious. This is the intracellular programmes and timers that run in developing precursor cells and change the cells over time. The molecular mechanisms underlying these programmes are largely unknown. My colleagues and I have studied such programmes in two types of rodent neural precursor cells: those that give rise to oligodendrocytes, which make myelin in the CNS (central nervous system), and those that give rise to the various cell types in the retina.

Introduction
The spectacular recent advances in developmental biology have drained a good deal of the mystery out of animal development. In some well-studied systems, for example, we now know many of the cell–cell interactions that help orchestrate development, as well as many of the extracellular signal molecules that mediate the interactions. We also know many of the gene regulatory proteins involved in cell specification and differentiation in these systems.

Nonetheless, there remain important areas of ignorance. Among these are the intracellular programmes that change developing animal cells over time and help to control the sequence and timing of developmental events; we know nothing about the molecular basis of most of these, largely because so little attention has been paid to them. Here, I review the intracellular developmental programmes that my colleagues and I have uncovered in the course of our studies of mammalian neurodevelopment.

Almost all of the work that I discuss was carried out by a remarkably talented group of postdoctoral fellows, Ph.D. students, and associates over about a 20-year period. The prize awarded to me rightly belongs to them.

An intracellular timer in oligodendrocyte precursor cells (OPCs)
Our interest in developmental timing began with a surprising experimental result obtained by Erika Abney, an immunologist originally from Mexico who joined our group in the late 1970s. We had earlier defined a set of cell-type-specific markers that allowed us to recognize the major types of glial cells found in the rat CNS (central nervous system): astrocytes, which are heterogeneous and have many functions; oligodendrocytes, which myelinate neuronal axons; and ependymal cells, which line the fluid-filled ventricles of the brain [1]. Erika determined when the first cells of each type appeared in the developing rat brain. Analysing brain cell suspensions from E11 (embryonic day 11) through to birth at around E21, she found that the first astrocytes appeared at E15–E16, the first ependymal cells at E17–E18, and the first oligodendrocytes at around the time of birth (E21–E22). Amazingly, when she isolated cells from E10 brain and cultured them in 10% FCS (foetal calf serum), the times of first appearance of these three cell types were the same as if the cells had been left in the developing brain; moreover, when cultures were prepared from E13 brain, all three cell types first appeared 3 days earlier, just as in vivo (Table 1) [2]. I find these results as shocking today as I did in 1981 when we published them, as the axial cues and morphogen gradients that play such an important part in controlling cell specification in early animal development were presumably lacking in these cultures. Moreover, it seemed unlikely that...
sequential cell–cell interactions could occur normally in such cultures to explain the preserved timing of initial glial cell development.

Whatever the nature of the timing mechanisms involved, the finding that they apparently could operate normally in dissociated–cell culture suggested that they might be accessible to study. The complexity of the brain cell cultures, however, would make such an analysis extremely difficult, which is why we switched to the developing optic nerve, which is one of the simplest parts of the CNS.

The optic nerve contains no neurons, although it contains the axons of retinal ganglion neurons; the main cell types are astrocytes and oligodendrocytes (Figure 1). We focused on the timing of oligodendrocyte development. Whereas the astrocytes in the optic nerve develop from the neuroepithelial cells of the optic stalk (the primordium of the nerve), the oligodendrocytes develop from precursor cells that migrate into the developing nerve from the brain beginning before birth [3]. The OPCs divide a limited number of times before they stop and terminally differentiate into myelin-forming oligodendrocytes. As in the brain, the first oligodendrocytes appeared in the rat optic nerve around the day of birth, and their numbers then progressively increased over the next 6 weeks. Our goal was to understand why the OPCs stopped dividing and differentiated into oligodendrocytes when they did. This was not known for any type of mammalian precursor cell, and this is still largely true today.

As was the case in embryonic brain cell cultures, we could reconstitute the normal timing of oligodendrocyte development in cultures of embryonic rat optic nerve cells, as long as there were sufficient numbers of astrocytes in the cultures to stimulate the OPCs to divide [4]. Mark Noble, at the Institute of Neurology in London, had shown that astrocytes produce a mitogen for OPCs, which is required to stimulate OPCs to proliferate and to prevent their premature differentiation [5]. Both he and our University College London colleague Bill Richardson showed independently that the mitogen is PDGFα (platelet-derived growth factor α) [6,7]. Our three laboratories collaborated to show that PDGF could bypass the need for large numbers of astrocytes and allow normal timing of oligodendrocyte development in sparse embryonic optic nerve cell cultures [8].

Sally Temple, a Ph.D. student, showed that OPCs have a cell–intrinsic mechanism that helps determine when they stop dividing and differentiate [9]. She placed single OPCs from P7 (postnatal day 7) rat optic nerve on to monolayers of astrocytes in individual microwells and found that the isolated single cells divided a variable number of times, with a maximum of eight divisions; the crucial finding was that all of the progeny of an individual OPC stopped dividing and differentiated at about the same time. She then showed that, even if the two daughter cells of an OPC were placed on astrocyte monolayers in separate microwells, they still tended to divide the same number of times and for the same period of time before they stopped dividing and differentiated, establishing that an intrinsic counting or timing mechanism is built into each OPC. We spent much of the next 15 years trying to determine how the cell–intrinsic mechanism operates. This seemed a worthwhile effort, as we assumed that a similar mechanism probably operates in many types of precursor cells.

Ben Barres, a postdoc from Harvard Medical School, advanced our study and understanding of the cell–intrinsic mechanism in two important ways. First, he developed a sequential immunopanning method to purify OPCs to homogeneity from neonatal rat optic nerves [10]. Secondly, he showed in serum-free clonal-density cultures of purified OPCs that the intrinsic mechanism depended on TH (thyroid hormone), as well as on PDGF [11]. Without PDGF, the cells prematurely stopped dividing and differentiated. In the presence of PDGF but without TH, most OPCs failed to stop dividing or differentiate; if TH was added after the time when most OPCs would have differentiated had the hormone been present all along, the cells quickly stopped dividing and differentiated. Nathalie Billon, a postdoc from France, and Yasu Tokomoto, a postdoc from Japan, later collaborated with Björn Vennström at the Karolinska Institute in Sweden to show that α1 TH receptors mediated this effect of the hormone on OPCs [12].

Ben’s findings suggested that OPCs were able to count divisions or measure time in the absence of TH, but TH was required for the cells to withdraw from the cell cycle and

Table 1 | Time of first appearance of three classes of glial cells in the developing rat brain and in cultures of embryonic rat brain cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>In vivo (E15–E16)</th>
<th>In culture of E10 brain</th>
<th>E13 brain (E21–E22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astrocytes</td>
<td>5–6 days</td>
<td>2–3 days</td>
<td></td>
</tr>
<tr>
<td>Ependymal cells</td>
<td>7–8 days</td>
<td>4–5 days</td>
<td></td>
</tr>
<tr>
<td>Oligodendrocytes</td>
<td>Around birth</td>
<td>13–14 days</td>
<td>10–11 days</td>
</tr>
</tbody>
</table>

Figure 1 | A simplified drawing of the vertebrate optic nerve

![Image of the vertebrate optic nerve with labels for retinal ganglion cell, Optic nerve, astrocyte, oligodendrocyte, and myelin.]
differentiated when the intrinsic mechanism indicated it was time. Therefore the counter or timer seemed to consist of at least two functional components: (i) a counting or timing component, which depended on PDGF but not on TH and counted cell divisions or measured elapsed time, and (ii) an effector component, which was regulated by TH and stopped cell division and initiated differentiation when the counting or timing component indicated it was time. Bögler and Noble reached a similar conclusion using a combination of PDGF and FGF2 (basic fibroblast growth factor) to keep the OPCs dividing beyond their normal limit [13].

Yasuo also showed that one or more members of the p53 family of proteins was required for TH to trigger OPC differentiation [14], and Nathalie, in collaboration with Gerry Melino and his colleagues in Rome, provided evidence that p53 and p73 were the relevant family members [15]; it remains to be discovered how these proteins function in OPC differentiation.

Why should TH regulate the intrinsic counting or timing mechanism in OPCs? Adult animals use hormones to help co-ordinate the behaviour of their cells throughout the body, and so it makes sense that developing animals use hormones such as TH to help co-ordinate the timing of development in their various organs and tissues. TH, for example, has been shown to co-ordinate the onset of myelination by oligodendrocytes and Schwann cells in the central and peripheral parts respectively of the developing auditory nerve [16].

Fen-Biao Gao, a postdoc from Duke University, used the Barres method to purify OPCs from embryonic rat optic nerve and showed that, in serum-free and extract-free clonal-density cultures containing PDGF and TH, the purified cells stopped dividing and differentiated apparently according to the same schedule as they do in vivo [17]. He also showed that, on average, OPCs purified from E18 optic nerve proliferated for longer under these conditions than did OPCs purified from P7 nerve [18], suggesting that the reason OPCs from P7 optic nerve went through a variable number of divisions before they differentiated [9] was that they varied in their stage of maturation. Most importantly, he showed that OPCs cultured at 33°C divided more slowly but stopped dividing and differentiated earlier, after fewer divisions, than when they were cultured at 37°C [19]. This finding provided strong evidence that the cell-intrinsic mechanism did not depend on counting cell divisions but instead measured time in some other way, leading us to refer to the mechanism as an intracellular timer. But what was the other way, and why did the timer run faster at the lower temperature?

Some protein components of the intracellular timer

Ian Hart, a training neurologist doing a Ph.D., had collaborated earlier with Bill Richardson to show that the intrinsic timing mechanism probably did not depend on changes in either the number of PDGF receptors on the surface of the OPC or the intracellular signalling pathways that the receptors activate. He showed that OPCs that had stopped dividing and begun to differentiate still retained large numbers of PDGF receptors [20], which could still be stimulated by PDGF to increase cytosolic Ca2+ [21] and to activate the transcription of immediate-early genes in the nucleus [22].

The first clue to the molecular nature of the intrinsic timer came from Béatrice Durand, a postdoc from Strasbourg. She showed that the amount of the Cdk (cyclin-dependent kinase) inhibitor p27 (p27Kip1) progressively increased in the nucleus of purified OPCs as they proliferated in the presence of PDGF and the absence of TH [23]. The amount of p27 protein reached a plateau at the time when most of the cells would have stopped dividing if TH had been present; without TH, however, the cells continued to proliferate, despite the high levels of p27. She also found that the level of p27 protein rose faster at 33°C than at 37°C, suggesting that this may have been at least part of the reason that the timer ran faster at the lower temperature. Béa then collaborated with Jim Roberts in Seattle, the head of one of the three laboratories that knocked the p27Kip1 gene out in mice [24–26]. She showed that, in cultures containing PDGF and TH, p27-deficient OPCs divided for 1–2 days longer than wild-type OPCs before they stopped dividing and differentiated, even though the cell-cycle times were indistinguishable [27]. This finding suggested that p27 is one component of the timer. Moreover, the p27-deficient cells were defective in both the timing and effector components of the timer, suggesting that p27 plays a part in both components. Jim Apperly, a Ph.D. student, showed that overexpression of p27 accelerated the timer, providing further support for a role of p27 in the timing process [28]. Yasu Tokumoto showed that p27 mRNA levels remained constant as the protein increased in proliferating OPCs [28], suggesting that the increase in the protein over time depended on post-transcriptional mechanisms, which remain to be identified.

As p27-deficient mice are approx. 30% larger than normal and have increased cell numbers in all of their organs, as a result of increased cell proliferation rather than decreased cell death [24–26], it seems likely that p27 plays a similar role in timing cell-cycle withdrawal and the onset of differentiation in many cell lineages. A homologue of p27 is present in Droso phila and Caenobrachidites elegans, and if the gene encoding it is inactivated by mutation in either organism, cells go through an extra division or two in several cell lineages [29–31]. Thus Cdk inhibitors are probably involved in stopping the cell cycle at the appropriate time during development in all animals.

The p27 protein, however, seemed to be only a minor component of the timer that operates in OPCs, as the timer still worked in p27-deficient OPCs; it just worked inaccurately. The phenotype of mice deficient in another Cdk inhibitor, p18 (p18ink4c), is very similar to that of p27-deficient mice [32]. Yasu Tokumoto found that this protein was expressed in OPCs and increased much like p27 as OPCs proliferated in culture, suggesting that it was also part of the timer; like p27, its increase was also controlled post-transcriptionally [28].

Toru Kondo, another postdoc from Japan, showed that Id4 (inhibitor of differentiation protein 4) was also a component...
of the timer, although it worked in the opposite way to p27 and p18 [33]. Id proteins inhibit basic helix–loop–helix gene-regulatory proteins that are required for the differentiation of many types of precursor cells; in this way, they inhibit differentiation and promote proliferation of the precursors. Toru found that Id4 decreased as purified OPCs proliferated in the presence of PDGF and the absence of TH and that, in this case, the Id4 mRNA and protein decreased in parallel, suggesting that a transcriptional mechanism was probably responsible for the progressive decrease in Id4 protein. He also showed that overexpression of Id4 prolonged OPC proliferation and inhibited differentiation in the presence of PDGF and TH. More recently, Toru collaborated with Fred Sablitzky at Nottingham University and Patrizia Casaccia-Bonnefil at Robert Wood Johnston Medical School in New Jersey to show that neural stem cells isolated from the brains of Id4-deficient mouse embryos produced oligodendrocytes prematurely [33a]. Taken together, these findings suggest that Id4 is another component of the timer and that its progressive decrease helps control when OPCs stop dividing and differentiate.

The OPC timer, like other intracellular timers, is still poorly understood. It is clear, however, that it is complex and depends on the progressive increase of some intracellular proteins such as p27 and p18 and the progressive decrease of others such as Id4. Both transcriptional and post-transcriptional controls have roles, but how these controls operate remains a mystery.

**An intrinsic maturation programme in OPCs**

The intracellular timer that helps control when OPCs withdraw from the cell cycle and differentiate seems to be only one part of a much more complex cell-intrinsic timing mechanism that changes many of the properties of OPCs over time.

Charles Ffrench-Constant, a medically trained Ph.D. student, found that there were small numbers of OPCs in cell suspensions prepared from adult rat optic nerves [34], suggesting that OPCs are present in the optic nerve throughout postnatal life. Mark Noble and his colleagues found these adult OPCs independently and characterized them in more detail [35]. Julia Burne, a technician turned Ph.D. student, collaborated with Barbara Fulton in the Anatomy Department at University College London, to visualize OPCs in the rat optic nerve at different times in development. They found that the cells became progressively more complex in morphology with age [36], but it was not clear whether these changes reflected changes in the cells’ environment with age, an intrinsic maturation programme operating within the cells themselves, or both.

Fen-Biao Gao provided strong evidence that perinatal OPCs, at least, had an intrinsic developmental programme that changed many aspects of the cell over time [18,23]. He first compared the properties of purified E18 OPCs with purified P7 OPCs (which are 10 days older) using time-lapse video recording of individual clones in culture. He found that, in serum-free cultures containing PDGF, but not TH, the embryonic OPCs had a simpler morphology than the P7 OPCs and divided and migrated faster; moreover, in the presence of PDGF and TH, the embryonic cells divided more times before differentiating than did the P7 cells (Table 2). Remarkably, when he cultured purified E18 OPCs in PDGF without TH for 10 days (so that they were now the same age as P7 OPCs), he found that the embryonic cells had acquired all the properties of the P7 cells (see Table 2), indicating that developing OPCs had an intrinsic maturation programme.

Dean Tang, a Chinese postdoc from Wayne State University, together with Yasu Tokumoto, showed that purified P7 rat OPCs could proliferate in serum-free culture for more than 1 year in PDGF without TH. After many months in culture, Dean found that the OPCs started to express the glycolipid galactocerebroside [37], which we originally believed was expressed only by oligodendrocytes and myelin in the CNS [38]. The galactocerebroside-expressing OPCs continued to proliferate and did not express other oligodendrocyte or myelin markers. The expression of galactocerebroside was not a culture artefact; Ben Barres and his colleagues at Stanford had shown earlier that OPCs in the rat optic nerve start to express galactocerebroside after many months *in vivo* [39]. Is it possible that the intrinsic maturation programme in OPCs continued to change the cells for months? This would be remarkable and would raise the question of how such an extended programme could work.

It is important to emphasize that the developmental programmes and timers that I have been describing in OPCs are not set in stone. As already mentioned, for example, most OPCs ignored the timer that helps determine when they stop dividing and differentiate if TH was omitted from the culture medium [11]. Moreover, Toru Kondo found that OPCs were not irreversibly committed to becoming oligodendrocytes or even glial cells: transient exposure of purified OPCs in culture to BMP4 (bone morphogenic protein 4), followed by FGF2, rapidly reprogrammed the cells to become more like multipotential CNS neural stem cells that could produce both neurons and glia [40]. Just 2 days in BMP4 was enough to induce the transcription of a variety of genes, including *sox-2*, that are normally expressed in neural stem cells. Toru, then in his own laboratory, focused on the induction of the

### Table 2 | Maturation of purified E18 rat OPCs in culture

<table>
<thead>
<tr>
<th></th>
<th>E18</th>
<th>P7</th>
<th>E18 + 10 days in vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell-cycle time</td>
<td>18 ± 1</td>
<td>27 ± 2</td>
<td>27 ± 3</td>
</tr>
<tr>
<td>Migration rate</td>
<td>30 ± 3</td>
<td>22 ± 2</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>Morphology</td>
<td>Simple</td>
<td>Complex</td>
<td>Complex</td>
</tr>
<tr>
<td>Proliferative capacity in PDGF and TH</td>
<td>++++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

*Data taken from [18]. Purified E18 or P7 OPCs, or E18 OPCs that were passaged after 10 days in culture, were cultured at clonal density in PDGF, but in the absence of TH. The cell-cycle times and migration rates are expressed as means ± S.E.M.*
A putative timer protein in silkworm

Before leaving intracellular timers, I want to mention one of the most remarkable reported examples of such a timer, which, as far as I can tell, has received little, if any, attention.

Many insects enter a period of developmental arrest, called diapause, in response to particular environmental conditions [42]. Early embryos of the silkworm *Bombyx mori* are an example: once they have entered diapause, they can be induced to restart development by exposure to cold (∼5 °C) for about 2 weeks. For more than 30 years, Professor Hidenori Kai of Tottori University in Japan has studied cold-induced termination of diapause in the silkworm. He originally found that, when diapausing eggs are exposed to cold, an enzyme activity, assayed as an esterase, turns on after about 2 weeks, which is right around the time that development restarts; the enzyme activity then rapidly shuts off again [43]. Remarkably, he found that the same sequence happens in a test tube when he cooled an extract of the diapausing eggs or a purified preparation of the enzyme (now shown to be an ATPase): the ATPase turns on after about 2 weeks at 5 °C and then quickly turns off again. Professor Kai calls the protein TIME, for time-interval-measuring enzyme, as it seems to measure the duration of the cold stimulus and thus acts as a diapause-duration timer [44]. How does a protein (or even a protein complex) in a test tube measure time over a 2 week period? He suspects that the protein undergoes a series of conformational changes. If correct, TIME would be an exceptionally interesting and challenging protein for structural biologists to study.

Developmental programmes in retinal precursors

As mentioned earlier, we studied the optic nerve because it is one of the simplest parts of the CNS. From time to time, however, we extended our studies into the retina, which is far more complex. We did this principally to address a fundamental question in developmental biology: how do cells diversify and become different from one another? The question is especially challenging to address in the CNS, where there are probably more molecularly distinct cell types than in the other parts of the body put together. The retina is an attractive part of the CNS in which to approach the problem, as it contains a manageable number of cell types (Figure 2), which develop on a predictable schedule, even in explant [45] or reaggregated cell [46] cultures.

A breakthrough in understanding cell diversification in the vertebrate retina came at the end of the 1980s, when three laboratories independently carried out cell lineage tracing studies, using either retroviral gene transfer in the rodent retina [47] or intracellular injection of tracer proteins in the frog retina [48,49]. These landmark studies showed that the two types of photoreceptors (rods and cones), the four types of neurons (amacrine, horizontal and bipolar interneurons, and ganglion cells – the only neurons whose axons project out of the retina) and the Müller glial cells (see Figure 2) can all develop from a single retinal precursor cell. The studies thereby identified the central question in retinal cell diversification: how do the multipotential precursors decide what type of retinal cell to become?

Takashi Watanabe, a neurosurgically trained postdoc from Tokyo University, began to address the question by studying the timing of rod photoreceptor development. Using anti-rhodopsin antibodies to unambiguously identify rods, he found that rods first appeared in small numbers in the rat retina at E20 and then increased rapidly for the next week or so. Although rhodopsin-positive rods failed to develop in dissociated cell cultures of E15 rat retina, they developed right on schedule if the E15 cells were centrifuged into a pellet that was then cultured on a floating polycarbonate filter. The pellet culture system allowed him to mix cells from different developmental ages. He labelled the DNA in proliferating E15 cells with BrdU (bromodeoxyuridine) and mixed the labelled cells with a 50-fold excess of unlabelled newborn retinal cells. The surprising finding was that the labelled E15 cells did not give rise to rods until the equivalent of E20, just as they did when cultured alone. Thus the presence of a vast excess of newborn cells, which were producing large numbers of rods from the start of the culture, did not affect the timing of rod production by the E15 cells in the same pellet [50]. These results strongly suggested that retinal precursors change their intrinsic developmental potential as development progresses.

On the other hand, although the presence of neonatal cells did not alter the time at which the E15 cells first gave rise to rods, they did increase the proportion of rods that the E15 cells generated [50], apparently by producing a short-range, rod-promoting, diffusible signal [51]. We concluded from these studies that a combination of cell–cell interactions and an intrinsic developmental programme in the precursor cells that changes a cell’s developmental potential over time contributes to retinal cell diversification [50], a view that is still widely held today [52].

One way to help determine the relative importance of intrinsic versus extrinsic factors in cell-fate choice is to culture precursor cells at clonal density under conditions where they proliferate and differentiate, so that every cell division, differentiation event and death can be followed. Abbie Jensen, a postdoc from the University of Wisconsin in Madison, developed the first such mammalian retinal cell culture system [53]. She was able to follow the development...
of mouse retinal precursor clones, either by continuous time-lapse video recording or by daily microscopic observation, and showed that, even when cultured in a homogeneous and constant environment where the cells could not contact cells outside their own clone, the precursor cells varied in their proliferative capacity, cell-cycle time and the cell types that they generated. Moreover, some individual precursor cells generated both neurons and glia, and the neurons developed before glial cells, just as in vivo.

Michel Cayouette, a postdoc from the University of Laval in Quebec, extended Abbie’s observations by developing a serum-free and extract-free clonal-density culture system to follow the fate of individual clones of proliferating E16–E17 rat retinal precursor cells. He compared the development of clones in these cultures with that of clones that developed in explant cultures of E16–E17 retina in 10% FCS; clonal development in such explant cultures had been shown to closely mimic clonal development in vivo [54]. Remarkably, he found that the precursor cells behaved similarly in both types of cultures: the clones that developed in the two cases were very similar, in both their size and the cell types that they contained. This finding was unexpected because the environments in the two cases were radically different; each precursor cell in an explant was not only surrounded by other cells in the developing neuroepithelium, but also was bathed in 10% FCS, whereas the dissociated precursor cells were cultured at clonal density without serum (but with the addition of several known growth factors) and could presumably only interact with cells within the same clone; moreover, in the clonal-density cultures, no one cell type seemed to be required for the development of any other cell type [55]. These surprising results challenged the current view of how cells diversify in the developing vertebrate retina, including our original model [50]. They suggested that positive inductive signals are unlikely to be important in determining cell-fate choices in the developing rat retina, at least from E16–E17 onwards. They suggested instead a model in which retinal precursor cells become specified some time before E16–E17 and then step through an intrinsic developmental programme, which can be different for different precursors [55]. If this model is correct, it will be important to determine when and how retinal precursors become programmed, how many programmes there are and how the programmes operate.

One aspect of the putative developmental programmes running in retinal precursor cells is especially surprising. Before Michel did his clonal culture experiments, he worked together with Alan Whitmore, a postdoc from the Institute of Ophthalmology in London, to investigate whether asymmetrical segregation of cell-fate determinants during mitosis...
of retinal precursor cells might play a part in retinal cell diversification. Such mechanisms were known to play a crucial part in cell diversification in invertebrates, but it was still uncertain how important they were in vertebrate development. Michel and Alan found for the first time that some retinal precursors in the newborn rat retina apparently divided asymmetrically to produce two molecularly distinct daughter cells: whereas most precursors divided horizontally, with their mitotic spindle oriented in parallel to the plane of the neuroepithelium, a minority divided vertically, with their spindle oriented at right angles to the epithelium [56]. They showed that mNumb protein, a mammalian homologue of the Drosophila cell-fate determinant Numb, was located at the apical pole of the precursor cells; thus, in vertical divisions, only the apical daughter cell would inherit mNumb, whereas in horizontal divisions both daughters would do so.

Michel then used time-lapse video recording of retinal precursors expressing GFP (green fluorescent protein) in explants of newborn rat retina to follow the fates of the two daughter cells produced by either vertical or horizontal division [57]. He showed that, independent of the plane of division, the two daughters rarely divided again, as expected at this relatively late stage of retinal development. The two daughters of horizontal divisions, however, almost always became photoreceptors, whereas the two daughters of vertical divisions almost always became different: usually one becoming a rod and the other either an interneuron (an amacrine or bipolar cell) or a Müller cell (see Figure 2). Thus, at this stage of retinal development, the plane of division clearly influenced cell-fate choice, and Michel provided indirect evidence that the asymmetrical segregation of mNumb in vertical divisions might be involved in the choice, possibly by inhibiting Notch signalling in the apical daughter cell, as has been shown for Numb in Drosophila (reviewed in [58]). Remarkably, Michel found that some precursor cells proliferating in dissociated cell culture of newborn rat retina also rotated their mitotic spindle through 90° just before dividing [55], consistent with his evidence that asymmetrical segregation of cell-fate determinants may be important for some late cell-fate choices and that these choices were made correctly in clonal-density dissociated cell cultures. If the pre-programming model is correct, the ability of a retinal precursor cell to re-orient its mitotic spindle and divide asymmetrically at a specific time in development can apparently be part of the programme.

There are other examples where neural precursor cells seem to be specified early and then step through a developmental programme. Sally Temple and her colleagues have provided compelling evidence for such programming in clonal cultures of mouse brain precursor cells. These cells undergo stereotyped patterns of cell division and differentiation to produce neurons and glial cells [59] in the same characteristic sequence as seen in vivo [60], and some of the precursors divide asymmetrically and segregate m-Numb preferentially to one daughter cell [61]. Neuroblasts in the Drosophila CNS are perhaps the best understood example of precursor cell programming. They go through a series of asymmetrical divisions to produce a variety of cell types in a predictable sequence, even in dissociated-cell culture [62,63], sequentially expressing different sets of transcription factors with each cell division [64]. These are likely to be the first complex intracellular programmes to be understood at the molecular level [65].

Conclusions

Intracellular programmes that change developing cells over time are among the least understood processes in animal development. They deserve much more attention than they have received so far and would be challenging subjects for bold biochemists.

I thank the students, postdocs and associates mentioned, as well as all of those not mentioned because their contributions fell outside the subject of this review, I owe them all more than I can possibly repay. I thank Avrion Mitchison, my only scientific mentor, for taking me in and showing me how. I am extremely grateful to the Medical Research Council for providing my salary and research costs for 31 years.

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