THE ORIGINS OF NEURONS IN THE CENTRAL NERVOUS SYSTEM

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The origins of neurons is basically similar in all regions of the central nervous system. Neurons are derived from a population of neuroepithelial precursor cells. Once generated, neurons cease dividing, commence differentiation, and migrate away from the neuroepithelium. In most mammals, neurogenesis is embryonic; there are a few exceptions though – cerebellum, hippocampus and olfactory bulb being the most studied – where neurons continue to be generated in the neonate. The central question to be addressed by those interested in neurogenesis is: what are the cellular and molecular mechanisms that control this process of differentiation? A further issue is whether these mechanisms are as similar in different regions of the central nervous system (CNS) as the superficial similarity would suggest. Addressing this question is complicated, however, by the fact that more is going on in the neuroepithelium than the generation of neurons. Brain cells are becoming specified in other ways; for example their positional specification is being determined. More particularly, in the context of this discussion, some cells are becoming specified to become glia, and the cells that take on this fate are also derived from the same neuroepithelium as neurons. How, then, is a cell steered down one pathway of differentiation rather than the other?

The mechanism of the steering process is still unknown. The pathway that cells are steered along, however, is beginning to come clearer, largely as a result of the recent interest in cell lineage. It is these studies that I shall principally consider here.

CELL LINEAGE

The generic experiment in a study of cell lineage is to label a precursor cell *in situ*, without disturbing its normal development, and see what it becomes. In general, there can be two alternative outcomes: either the cell will generate progeny with a single fate, however fate is defined, or it can take on multiple fates. Thus, the progeny can all become neurons, all astrocytes, or all oligodendrocytes; or they can become a mix of the different cell types. Similarly, they can all form part of the same neural structure or disperse among several. If the progeny take on multiple fates, then the labelled precursor cell must have been multipotential, at least with regard to that particular aspect of cell fate. If the progeny all take on a single fate, then the precursor cell *might* have had a more limited potential. The reservation in that statement is important; there are numerous reasons why a cell might be multipotential yet still generate cells with a single fate. For example, cells of one type might be predominantly in demand at a particular point in development, so the cell would be overwhelmingly pushed into that particular fate. Even if one can be fairly sure that stoichiometric arguments of this sort do not apply, the conclusion can be drawn only that the cell was specified to take on the restricted fate, not that it was fully determined. Were it transplanted to another situation, it might demonstrate a broader potential than observed *in situ*. These limitations notwithstanding, cell lineage is an important first step towards understanding developmental decisions. It helps narrow down when the decisions are made, and what restrictions the precursor cells are undergoing.

There have been two key methods employed to study cell lineage in the vertebrate nervous system: retroviral vectors[1,2] and the iontophoretic injection of fluorescent dyes[3–5]. Each has its advantages and disadvantages, but briefly, retroviruses are genetic, indelible and widely applicable; fluorescent dyes are more sure in terms of the identity and position of the cells labelled, but are more short-lived and not so widely applicable. These two methods between them have been applied to several different regions of the CNS. I will consider two, the retina and the cerebrum, in an attempt to ask whether a single coherent picture is emerging as to how neurogenesis might be controlled.

THE CEREBRUM AND RETINA COMPARED

Cell lineage in the retina has been studied in rodents using retroviruses[6,7] and in frogs by dye injection[3,4]. The results of both sets of studies have been largely similar: retinal

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precursor cells generate multiple cell types. This is true whether they are labelled early or late in development. Indeed, cells can be labelled just prior to the final or penultimate divisions and still generate multiple cell types[7]. The main exception to this rule can apparently be explained by stoichiometry. Many precursor cells labelled late in development generate rod photoreceptors exclusively, but this is at a stage in development when the vast majority of cells produced in the retina are rods. Thus whatever the potential of the precursor cells, most will generate only rods.

These data have been interpreted to mean that the retinal precursor cells are multipotential; that right up to their final divisions, they have the capacity to produce all the cell types being generated in the retina at that time. (The exception is retinal astrocytes which are emigrés from the optic nerve[8].) Needless to say, this does not mean that their potential does not change as development proceeds. Early on, for example, this potential will include the capacity to generate ganglion cells, an early cell type, whereas later the precursor cells, though still multipotential, no longer generate this cell type. The implication is, therefore, that cell fate is determined on a cell-by-cell basis. As each cell becomes post-mitotic, its fate is determined, presumably by interactions with the immediate environment. There are data which support such a model of fate determination[9–11].

It cannot be quite that simple, though. Williams and Goldwitz[12] have pointed out that the numbers do not fit such a purely stochastic model. The proportion of pure clones of one cell type, and restricted clones of just two cell types, is too high to fit such a model. There is, in other words, a greater tendency than predicted by chance for a precursor cell to continue to produce a given cell type once it has begun to do so. I will consider below what this might mean.

The retroviral approach when applied to the cerebrum has given different results[13–16]. For the most part, labelled precursor cells generate clusters of cells, all of which are of one type. The precise number of types of cluster is not totally clear, but combining the results from different laboratories suggests a figure of six: neuronal clusters are of two types (either pyramidal or non-pyramidal); glial clusters are of three types (oligodendrocyte, white matter astrocyte and grey matter astrocyte); and there are also clusters of an unidentified astrocyte-like cell type. A number of the clusters, however, are composed of both neurons and glial cells. In the retroviral studies, neurons and oligodendrocyte clusters have been described both *in vivo*[13,15] and in cultures of cerebral cells[17]. Neuron and astrocyte clusters have not yet been formally identified using the retroviral method, but have been hinted at by Walsh and Cepko[18] and have been identified in culture after single cell cloning procedures by a number of groups[19–21].

This body of data is much more difficult to interpret than the retina data for a variety of reasons. The first is that, as already discussed, a precursor cell can be multipotential yet still generate a single cell type. The second problem is caused by a technical limitation. Labelled clones in the retina were very easy to identify because they remained as discrete clusters of cells. This is not the case in the cerebrum, where clones tend to disperse. The precise extent of the dispersion is not yet clear, although it may be very great indeed[18]. This raises the possibility that the clusters of cells of different types might themselves be related; one infected cell could give rise to more than one cluster. It is not yet clear how this possibility will affect the general conclusion that most cerebral precursor cells generate a single cell type.

To some extent both of these problems have been overcome by studies done in tissue culture[17]. Embryonic cerebrum can be dissociated into single cells, infected with retrovirus, and grown in monolayer culture. The infected precursor cells will divide and generate differentiated progeny, and just as *in vivo*, an analysis of the clones will reveal whether the progeny comprised one or many cell types. Since the normal tissue environment is disrupted in such an experiment, however, the outcome may well differ from what would have happened *in vivo*. Thus if the precursor cells were truly multipotential they might well generate a broader range of cell types than they managed *in vivo*. Moreover, since histogenesis is absent in culture, the problem of clonal dispersion is considerably reduced.

The result of such culture studies is largely similar to those *in vivo*, namely, the majority of clones are composed of a single cell type. The principal exception is a type of clone composed of neurons and oligodendrocytes and, as already noted, this is also the most common exception *in vivo*. This result suggests that a large proportion of cerebral precursor cells are restricted to a single fate. Lest any confusion arise, it should immediately be said that in positional terms the precursor cells seem to be multipotential; as far as has been determined, they contribute cells to multiple cortical layers and areas[18,22–24]. This confirms the earlier point: when referring to a cell as multipotential or specified, one must be precise about the aspect of phenotype that is being considered.

The conclusion that most cerebral precursor cells are restricted, immediately begs a further question: When and from what source do the restricted precursor cells themselves arise? Presumably, there is an earlier pluripotential cell type that generates everything, but the other combinations – the neuron/oligodendrocyte clones, the neuron/astrocyte precursor cells – must fit into the developmental profile also. These problems are not resolved, although we do now have unpublished evidence for a precursor cell type that has some of the properties expected of an early multipotential precursor cell type. The resolution of this early part of the cerebral lineage tree is one of the major unanswered questions in this field.

A UNIFYING THEORY?

We have considered two basically similar regions of the CNS – the cerebrum and the retina – and arrived at two

apparently disparate sets of results. In the retina, precursor cells are multipotential and cell type decisions are apparently made late; in the cerebrum, the majority of precursor cells seem to be restricted early in neurogenesis. Can we somehow convert this disparity into a neat unifying theory? I fear not, but the dissonance rests to some extent on semantics. There is a feature that links our data on the cerebrum[15,17] and the Williams and Goldowitz[12] perspective on the Turner and Cepko[6] data in the retina. The retina data say two things: first, that all conceivable combinations of cell types are found together in clones. This argues strongly for multipotency, and against any stepwise specification of precursor cells into restricted subpopulations. The second point, however, is that there are more clones with restricted progeny – one or two cell types – than would have been predicted by a strictly stochastic mechanism of fate determination. In other words, a precursor cell that has generated a cell of a particular type is more likely to continue to generate that type than any other, even though (as we have observed) the precursor cell seems to be multipotential.

It seems to me that this lineage inertia is unsurprising given the types of mechanism, that are likely to dictate cell fate. Simplistically, we might envisage a precursor cell being switched to generate cells of a particular fate by the ambient levels of a particular growth factor. When the tissue requires that particular cell type, the levels of the growth factor will rise until a threshold is crossed and the switch is thrown. Even though the overall level of the factor may subsequently fall, we might expect the precursor to retain its specified fate for a period because: (1) the switch is not reset until a second lower threshold is crossed; or (2) a second factor is required to switch fate to a different cell type; or (3) the local level of the factor is variable, so that some areas of tissue still have suprathreshold levels of the factor, even though the overall level is sub-threshold. Thus although the overall system is dynamic, there will be a tendency for individual precursor cells to be laggardly in keeping up with what is required. This simplistic model is almost certainly inadequate, but this type of inertia is surely going to be a feature of any such dynamic system.

How does this help unify the data from the retina and the cerebrum? So far I have described the precursor cells in these two issues in quite different terms: multipotential in the retina, specified in the cerebrum. If we think in terms of inertia, however, this difference amounts to either a small resistance to changes of fate (in the retina) or a large resistance (in the cortex). Thus, by saying that the cortical precursor cells are specified, we mean that they require a considerable shove to switch their fate, compared with the relatively small push that is required by retinal cells. This way of looking at lineage not only helps us unify the two sets of data, it also makes some sense of the mixed clones (e.g. neurons and oligodendrocytes) that are found in the cortex, because we would predict that some cells would change fate, great though the inertia might be.

This tentative model brings us to the realisation that to understand the process of fate determination further, we need to understand more about mechanism. This is currently very poorly understood. It is, I think, fair to say that we currently have little idea of what determines a cell to take on a particular neural fate, although a recent study with *myc* genes implies that they might play an important role[25]. The elucidation of mechanism must lie at the heart of further research in this area.

I acknowledge the financial support of the Medical Research Council, the Multiple Sclerosis Society of Great Britain and Northern Ireland, the Commission of the European Communities, and the British Council.

Key words: Cell lineage, Retina, Cerebral cortex, Retrovirus.

REFERENCES

1. Sanes JR, *et al.* Use of a recombinant retrovirus to study post-implantation cell lineage in mouse embryos. EMBO J 1986;5:3133–42.
2. Price J, *et al.* Lineage analysis in the vertebrate nervous system by retrovirus-mediated gene transfer. Proc Natl Acad Sci USA 1987;84:156–60.
3. Wetts R, Fraser SE. Multipotential precursors can give rise to all major cell types of the frog retina. Science 1988;239: 1142–5.
4. Holt CE, *et al.* Cellular determination in the *Xenopus* retina is independent of lineage and birth date. Neuron 1988;1: 15–26.
5. Fraser S, *et al.* Segmentation in the chick embryo hindbrain is defined by cell lineage restrictions. Nature 1990;344: 431–5.
6. Turner D, Cepko C. cell lineage in the rat retina: a common progenitor for neurons and glia persists late in development. Nature 1987;328:131–6.
7. Turner DL, *et al.* Lineage-independent determination of cell type in the embryonic mouse retina. Neuron 1990;4:833–45.
8. Watanabe T, Raff MC. Retinal astrocytes are immigrants from the optic nerve. Nature 1988;352:834–7.
9. Adler R, Hatlee M. Plasticity and differentiation of embryonic retinal cells after terminal mitosis. Science 1989;243: 391–3.
10. Reh TA, Tully T. Regulation of tyrosine hydroxylase-containing amacrine cell number in larval from retina. Dev Biol 1986;114:463–9.
11. Reh TA. Cellular interactions determine neuronal phenotypes in rodent retinal cultures. J Neurobio 1992;23: 1067–83.
12. Williams RW, Goldowitz D. Lineage versus environment in embryonic retina: a revisionist perspective. Trends Neurosci 1992;15:368–73.
13. Price J, Thurlow L. Cell lineage in the rat cerebral cortex: a study using retroviral-mediated gene transfer. Development 1988;104:473–82.
14. Luskin MB, *et al.* Cell lineage in the cerebral cortex of the mouse studied *in vivo* and *in vitro* with a recombinant retrovirus. Neuron 1988;1:635–47.
15. Grove EA, *et al.* Multiple restricted lineages in the embryonic cerebral cortex. Development 1993;117:553–61.
16. Parnavelas JG, *et al.* Separate progenitor cells give rise to pyramidal and non-pyramidal neurons in the rat telencephalon. Cerebral Cortex 1992;1:463–8.
17. Williams BP, *et al.* The generation of neurons and oligodendrocytes in the cerebral cortex from a common precursor cell. Neuron 1991;7:685–93.
18. Walsh C, Cepko CL. Widespread dispersion of neuronal clones across functional regions of the cerebral cortex. Science 1992;255:434–40.

19. Temple S. Division and differentiation of isolated CNS blast cells in microculture. Nature 1989;340:471–3.
20. Kilpatrick TJ, Bartlett PF. Cloning and growth of multipotential neural precursors: requirements for proliferation and differentiation. Neuron 1993;10:225–65.
21. Reynolds BA, *et al.* A multipotent EGF-responsive striatal embryonic pregenitor cell produces neurons and astrocytes. J Neurosci 1992;12:4565–74.
22. Price J, *et al.* Cell lineage in the cerebral cortex. Development, Suppl 2 1991:23–8.
23. McConnell SK, Kaznowski CE. Cell cycle dependence of laminar determination in developing neocortex. Science 1991;254:282–5.
24. Grove EA, *et al.* Neuronal precursor cells in the rat hippocampal formation contribute to more than one cyoarchitectonic area. Neuron 1992;8:217–29.
25. Bernard O, *et al.* L-*myc* and N-*myc* influence lineage determination in the central nervous system. Neuron 1992;9: 1217–24.