iBiology.org Teaching Tools
Martin Raff's Lecture Part 2:
Regulation of Cell Number

Teaching Tools were prepared by Martin Raff.

Contents

1. Keywords and Terms
2. Recommended Reading
3. Review Questions
4. Answers to Review Questions
5. Discussion Questions
6. Answers to Discussion Questions
7. Explain or Teach These Concepts to a Friend
8. Research the Literature on Your Own

---

1. Keywords and Terms

Oligodendrocytes, oligodendrocyte precursor cells (OPCs), apoptosis, glial growth factor (GGF), platelet-derived growth factor (PDGF), astrocytes, cellintrinsic timer, thyroid hormone, cyclin-dependent-protein kinase (Cdk) inhibitors, p27, inhibitor of differentiation (Id) protein, Id4

2. Recommended Reading

Molecular Biology of the Cell, Alberts et al., 5th edition: pp1108-1111

Essential Cell Biology, Alberts et al., 3rd edition: pp 643-645

Raff, M.C. (2006) The mystery of intracellular developmental timers. *Biochem. Soc. Transactions* 34: 663-670.

References

Raff, M.C. (1992) Social controls on cell survival and cell death. *Nature* 356: 397–400.

Barres, B.A., and Raff, M.C. (1999) Axonal control of oligodendrocyte development. *J. Cell Biol.*147: 1123–1128.

Raff, M. (2007) Intracellular developmental timers. *Cold Spring Harbor Symposia on Quantitative Biology, Symposium 72*: 431-435.

Gao, F., Durand, B., and Raff, M. (1997) Oligodendrocyte precursor cells count time but not cell divisions before differentiation. *Curr. Biol*. 7: 152–155.

Durand, B., and Raff, M.C. (2000) A cell-intrinsic timer that operates during oligodendrocyte development. *BioEssays* 22: 64–71.

Kondo, T., and Raff, M. (2000) The Id4 HLH protein and the timing of oligodendrocyte differentiation. *EMBO J.* 19: 1998–2007

3. Review Questions

1. What cell processes determine the number of cells in an organ?
2. What is the main function of oligodendrocytes?
3. What makes the optic nerve one of the simplest parts of the central nervous system?
4. What determines the final number of oligodendrocytes in the optic nerve?

5. What is the evidence that OPCs and oligodendrocytes need signals from other cells in order to avoid apoptosis?
6. What might be the advantage for animals to have such a ‘death by default’ mechanism, whereby their cells need constant signaling from other cells to avoid apoptosis?
7. Most cells in your body develop from precursor cells that divide a limited number of times before they stop dividing and differentiate into specialized cells, which usually do not divide again. How does this apply to oligodendrocyte development?
8. Some OPCs in the developing optic nerve divide more than other OPCs in the same nerve? What is the most likely explanation for these differences? What is the evidence for this explanation?
9. What is the function of p27, and what is the evidence that it is part of the cell-intrinsic timer that helps determine when OPCs stop dividing and differentiate?
10. How does the Id4 protein help control when OPCs stop dividing and differentiate?

4. Answers to Review Questions

1. The total number of cells in an organ depends on cell proliferation, cell death, and the migration of cells into and out of the organ.

2. They wrap around axons in the central nervous system of vertebrates to make an insulating myelin sheath, which speeds up the conduction of action potentials along the axon.

3. It contains no nerve cells, although it does contain the axons of retinal ganglion cells passing from the eye to the brain.

4. It depends on the number of oligodendrocyte precursor cells (OPCs) that migrate into the nerve from the brain during development, the number of times each OPC divides, and the number of OPCs and oligodendrocytes that die by apoptosis.

5. When single or purified OPCs or oligodendrocytes are cultured on their own in the absence of extracellular signal molecules, they undergo apoptosis. If, however, they are cultured in the presence of astrocytes or nerve cells, respectively, they survive.

The astrocytes and nerve cells produce a variety of survival-promoting signal molecules that signal the OPCs or oligodendrocytes, respectively, not to kill themselves; these signals inhibit the apoptosis suicide program that is present in almost all animal cells.

6. Having cells depend on survival signals produced by other cells could provide a powerful mechanism for ensuring that cells only survive where and when they are needed. In this way, for example, cells that end up in the wrong place might not receive the survival signals they need and consequently kill themselves by undergoing apoptosis. It also could provide a useful way to regulate cell numbers, as addressed in some facilitator questions below.

7. Oligodendrocytes develop from OPCs that divide a limited number of times before the stop dividing and differentiate into oligodendrocytes, which do not divide again.

8. It is thought that the differences reflect differences in OPC maturation, with the younger OPCs dividing more than the older OPCs. The evidence for this view is that OPCs isolated from embryonic optic nerves divide, on average, more times before they stop and differentiate than do OPCs from postnatal rat optic nerves.

9. p27 inhibits cyclin-dependent protein kinases that promote progress through the G1 phase of the cell cycle, thus acting as a brake on cell proliferation. As OPCs proliferate, the level of p27 protein progressively increases, and it is thought that this increase helps the OPCs stop dividing and differentiate. If recombinant DNA technology is used to over-express p27 in OPCs, the cells stop dividing and differentiate prematurely; if the gene encoding p27 is inactivated in OPCs, the cells go through 1 or 2 more divisions, on average, before they differentiate. In addition, P27 protein increases faster at 33°C than at 37°C, and the timer runs faster at the lower temperature. Together, these findings suggest that p27 is part of the timing mechanism that helps determine when OPCs stop dividing and differentiate.

10. Id proteins inhibit the function of basic helix-loop-helix transcription factors that are required for many types of precursor cells to stop dividing and differentiate. Id4 seems to serve this function in OPCs. The level of Id4 progressively decreases as OPCs proliferate, which is thought to help the cells to stop dividing and differentiate.

5. Discussion Questions

1. What is the neurotrophic hypothesis, and how could the mechanism proposed serve to regulate nerve cell numbers in the nervous system.
2. How does oligodendrocyte death during optic nerve development help match the number of oligodendrocytes to the number of axons in the nerve?
3. What is the evidence that oligodendrocyte death during optic nerve development helps adjust oligodendrocyte numbers to the number of axons in the nerve?
4. What is the evidence that the neuregulin GGF is one of the axon-derived survival signals for oligodendrocytes in the developing rodent optic nerve?
5. What is the evidence that OPCs have a cell-intrinsic mechanism that helps determine when they stop dividing and differentiate?
6. What is the reason for calling the mechanism that helps determine when OPCs stop dividing and differentiate a cell-intrinsic timer rather than a counter?
7. What is the evidence that the cell-intrinsic timer in OPCs is regulated by extracellular signals from other cells?
8. What is the role of thyroid hormone in oligodendrocyte development?
9. What might be the advantage of having a hormone regulate a cell-intrinsic timer like the one that operates in OPCs?
10. Both p27 and Id4 seem to be part of the intrinsic timer in OPCs. What happens to their levels as OPCs proliferate? How are these levels controlled, and how do we know?
11. We are about 3000 times larger than a mouse. What cell mechanisms might explain this difference in size?

6. Answers to Discussion Questions

1. The hypothesis has several parts. First, many developing nerve cells require survival signals (neurotrophic factors) released from the target cells they connect to. Second, in many cases, more nerve cells are produced than can be supported by the limited

amount of survival factors released by the target cells; therefore, some nerve cells receive insufficient amounts of the survival signal to avoid cell death. Third, this strategy of nerve cell overproduction followed by culling helps to ensure that all target cells are contacted by nerve cells and that extra nerve cells are automatically eliminated; in this way, the number of nerve cells is automatically adjusted to the number of target cells they connect to.

2. It works in much the same way as described above for nerve cells. Oligodendrocytes are overproduced, and about 50% of them die by apoptosis during normal optic nerve development. Newly formed oligodendrocytes seem to require survival signals from axons to avoid apoptosis; it is thought that the survival signals are on the axon surface, so that those oligodendrocytes that fail to contact an axon die by apoptosis. In this way, the number of oligodendrocytes is automatically adjusted to the number of axons.)

3. If the newborn optic nerve is cut behind the eye, the axons rapidly degenerate, and most of the oligodendrocytes die by apoptosis. If the number of axons is experimentally increased in the developing nerve (using genetic manipulations), more survival factor is available and fewer oligodendrocytes die, and so their number increases to match the increase in axons. If the number of oligodendrocytes produced is experimentally increased, all the extra cells die by apoptosis, as the axons can only support the survival of the normal number of oligodendrocytes. If the number of oligodendrocytes produced is experimentally decreased, the competition for axon-derived survival signals is decreased, and so fewer oligodendrocyte die, and their number ends up being normal.

4. There are several lines of evidence. First, GGF is present on the axon surface. Second, oligodendrocytes undergo apoptosis when cultured on their own, but GGF allows them to survive. Third, neurons can also support the survival of purified oligodendrocytes in culture, and anti-GGF antibodies block this survival-promoting effect of the neurons, indicating that neuron-derived GGF is responsible for the survival. Fourth, experimentally increasing GGF in the developing optic nerve decreases oligodendrocyte apoptosis, whereas decreasing GGF in the developing nerve increases oligodendrocyte apoptosis.

5. When an individual OPC is followed in culture, all of its progeny cells tend to stop dividing and differentiate at the same time. Moreover, when the two daughter cells of an OPC division are separated and cultured on separate beds of astrocytes (which

provide both survival factors and mitogens for the OPCs), the two daughters tend to divide the same number of times before they stop and differentiate.

6. When purified OPCs are cultured at 33°C rather than at the normal temperature of 37°C, the cells proliferate more slowly than they do at 37°C, but they stop dividing and differentiate sooner, after fewer divisions. This suggests that the cell-intrinsic mechanism does not operate by counting cell divisions but, instead, measures time in some other way—for example, by the increase in p27 protein, which occurs faster at 33°C than at 37°C.

7. When OPCs are cultured in the absence of the mitogen PDGF, they stop dividing immediately and differentiate into oligodendrocytes, indicating that the cell-intrinsic timer requires PDGF, which is normally produced by astrocytes, to operate normally, at least in culture. Moreover, if OPCs are cultured in PDGF but in the absence of thyroid hormone, most fail to stop dividing and differentiate when they would normally do so, indicating that thyroid hormone is also required for the OPC timer to work normally.

8. As indicated in the answer to Question 6, thyroid hormone seems to be required for OPCs to stop dividing and differentiate on their normal schedule. The hormone does not seem to be required for the cell-intrinsic timer in OPCs to keep time, because if the cells are cultured with PDGF but no thyroid hormone for a week or so and then the hormone is added, the cells stop dividing and differentiate quickly, indicating that the cells had been keeping time in the absence of the hormone; OPCs seem to need the hormone to stop dividing and differentiate when the timer indicates it's time.

9. Some hormones, including thyroid hormone, increase progressively during animal development and can therefore help to synchronize the timing of developmental events in different organs. It would be risky to allow cell-intrinsic timers in different organs to operate autonomously, as it is important that the development of different cell lineages in different organs be co-ordinated in the animal as a whole.

10. p27 protein level progressively increases, while Id4 protein level progressively decreases. The p27 protein level is controlled post-transcriptionally, as p27 mRNA level doesn't change as the level of p27 protein increases. It is not known which post-transcriptional mechanisms are responsible for the rise of p27 protein as OPCs

proliferate. Id4 protein is probably regulated transcriptionally, as the protein and mRNA levels decrease in parallel as OPCs proliferate.

11. We are 3000 times larger than mice because we have about 3000 times more cells than a mouse. In principle, it could be that we have so many more cells because fewer of our cells die by apoptosis during development, but this seems not to be a major explanation. We have more cells because our cells, on average, divide more times than do mouse cells. This is not because human cells divide faster than mouse cells; it's because our cells, on average, divide for longer than do mouse cells. This could be because the cell-intrinsic timers that operate in our precursor cells to limit proliferation are programmed to permit the cells to divide for longer than mouse cells, or it could be that the extracellular signals that stimulate cell proliferation are present for longer in developing humans than in developing mice; or, perhaps both mechanisms operate.

7. Explain or Teach These Concepts to a Friend

1. Explain how OPC proliferation is controlled in the developing optic nerve.
2. Explain the role of apoptosis in controlling oligodendrocyte and nerve cell numbers.
3. Explain how humans grow to be so much larger than a mouse.

8. Research the Literature on Your Own

1. What are the roles of cyclin-dependent protein kinase inhibitors in animal development?
2. What are the roles of Id proteins in animal development?
3. What are the roles of thyroid hormone in animal development?
4. What are the roles of cell-intrinsic timers in animal development?
5. What are the roles of apoptosis in animal development?