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# Martin Raff's Lecture Part 1:
# Regulation of Cell Size

Teaching Tools were prepared by Martin Raff.

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## 1. Keywords and Terms

Schwann cells, growth factor, mitogen, insulin-like growth factor 1 (IGF1), glial growth factor (GGF), cell-cycle checkpoint, aphidicolin, cyclins, cyclin-dependent kinases (Cdks), receptor tyrosine kinases, intracellular signaling pathways, MAP kinase, PI 3-kinase/Akt signaling pathway

## 2. Recommended Reading

1. Molecular Biology of the Cell, Alberts et al., 5th edition: pp 1108-1111

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

3. Conlon, I., and Raff, M.C. (1999) Size control in animal development. *Cell* 96: 235–244.

## References

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## 3. Review Questions

1. What parameters mainly determine the size of an animal or one of its organs?
2. Is a human bigger than a mouse largely because it has bigger cells or more cells, or does cell size and cell number contribute about equally?

3. Why does life depend as much on cell growth (cell enlargement) as it does on cell division?
4. What is a cell-size checkpoint in the cell cycle?
5. How do IGF1 and GGF differ in their effects on rat Schwann cells?
6. GGF but not IGF1 greatly increases the amount of the G1 cyclins (cyclin D1 and cyclin D2) in rat Schwann cells. Does this make sense, given the different effects that GGF and IGF1 have on these cells?
7. IGF1 and GGF both bind to the same class of cell-surface receptors, called receptor tyrosine kinases, and yet they have different effects on rat Schwann cells. From what you heard in my lecture, can you suggest why this might be so?
8. When proliferating rat Schwann cells are cultured in either a high concentration or a low concentration of GGF, but in a fixed concentration of IGF1, the cells in high GGF are, on average, smaller than those in low GGF. Why do you think this is the case?
9. When rat Schwann cells are cultured in 3% fetal calf serum (FCS, which contains growth factors and mitogens) in the presence of aphidicolin (which blocks DNA replication), the cells arrest in S phase but continue to grow, getting progressively larger each day. One can then compare the cell growth rates (measured by the amount of either volume or protein that a cell adds each day) of cells after one day, which are relatively small, with cells after a week, which are much larger. Remarkably, one finds that the growth rates are the same for the small and larger cells. Does this necessarily mean that the small cells and big cells produce the same amount of protein each day?
10. The cells in an animal require extracellular signals from other cells: they require growth factors to grow and mitogens to divide. Yeast cells, by contrast, do not require such signal proteins: they grow and divide in the presence of nutrients (such as sugars and amino acids) alone. Can you think of a rational explanation (in terms of evolution) for this difference?

## 4. Answers to Review Questions

1. The total number of cells, the size of the cells, and the amount of extracellular materials.

2. Mammals differ in size largely because of differences in cell number. A human weighs roughly 3000 times more than a mouse and has roughly 3000 times more cells.

3. All living things are either cells or made of cells, and cells can only be produced by the division of pre-existing cells. If cells divided without growing, however, they would get smaller and smaller, and there could be no increase in cell mass. Thus, cell growth is as important as cell division for maintaining life on Earth.

4. It is a hypothetical point in the cycle where a proliferating cell can pause and somehow assess whether it is large enough to proceed to the next step in the cycle; if it is not, the cell will wait until it has grown more before proceeding. It remains controversial whether animal cells have such checkpoints, which could help coordinate cell growth with cell division in order for proliferating cells to maintain an appropriate size.

5. IGF1 on its own stimulates Schwann cells to grow (enlarge), but it does not promote progress through the cell cycle. GGF has the opposite effect. Thus, IGF1 is a true growth factor for these cells, whereas GGF is a mitogen but not a growth factor for them. IGF1, however, can enhance the effect of GGF in stimulating cell-cycle progression.

6. Yes. The G1 cyclins are required to activate the cyclin-dependent protein kinases (Cdks) that promote progress through the G1 phase of the cell cycle into S phase. Since GGF but not IGF1 stimulates such progression in rat Schwann cells, it makes sense that GGF but not IGF1 increases G1 cyclins in these cells.

7. Whereas IGF1, but not GGF, binding to its receptors on rat Schwann cells causes a marked and sustained activation of the intracellular signaling kinase called Akt, GGF, but not IGF1, binding to its receptors on these cells causes a marked and sustained activation of different intracellular signaling kinases called Erk1 and Erk2, which are MAP kinases. This suggests that the PI 3-kinase/Akt signaling pathway is important for promoting cell growth, whereas the MAP kinase signaling pathway is important for promoting progress through the cell cycle.

8. GGF accelerates the rate at which Schwann cells progress through the cell cycle. Therefore, the cells in high GGF go through the cycle faster than cells in low GGF.

Because the concentration of IGF1 is the same in both conditions, the cells grow at a similar rate in both conditions. Thus, the cells in high GGF have less time to grow before they divide and therefore divide at a smaller size than cells in low GGF.

9. No. In fact, when the rates of protein synthesis are compared, the big cells are found to make protein at a higher rate than the small cells. The big cells and small cells grow at the same rate because the big cells also degrade (and, perhaps, secrete) proteins at a greater rate than the small cells.

10. Yeast cells are single-celled organisms, and so it is in the cell's interest to grow and divide as fast as it can, independent of other yeast cells. It would be disastrous for an animal if any of its cells grew and divided as fast as they could, as happens in some cancers. In normal animals, a cell only grows and divides when other cells stimulate it to do so; in this way, each cell's behavior is controlled and co-ordinated for the good of the animal as a whole.

## 5. Discussion Questions

1. The growth hormone (GH)/ IGF1 endocrine system is a major stimulator of mammalian growth: without treatment, babies that are unable to make GH end up as midgets, while those that produce excessive amounts of GH end up as giants. Could differences in this endocrine system account for why humans grow to be so much larger than mice? Explain your answer.
2. Since the growth of a cell depends on extracellular growth factors produced by other cells, is it likely that differences in the concentrations of such growth factors explain why some cell types in your body (such as skeletal muscle cells) are very large, while other cell types (such as lymphocytes) are very small?
3. Most of your cells are said to be diploid because they contain two complete sets of chromosomes, one set inherited from your mother and one set from your father. A general finding is that cell size increases with increasing ploidy: for example, tetraploid cells, which contain four complete sets of chromosomes, are about twice the size of diploid cells of the same kind. Can you think of reasons why cell size increases with increasing ploidy?

4. What does the drug aphidicolin do, and why is it useful for studying the growth (enlargement) of cells in culture?

5. Surprisingly, aphidicolin-arrested Schwann cells that are stimulated to grow by the growth factors present in FCS grow at a rate that is independent of cell size—that is, little cells and big cells add the same amount of protein or volume per day. Yeast cells arrested in S phase because of a mutation in a gene required for DNA replication also continue to grow when cultured in optimal nutrients. But, in contrast to aphidicolin-arrested Schwann cells (which are also arrested in S phase), the mutant yeast cells grow progressively faster as they increase in size. Can you suggest a possible explanation for this difference between the aphidicolin-arrested Schwann cells and the arrested mutant yeast cells, assuming that the difference is not caused by the different ways that the cells have been arrested in S phase?

6. Cancer results when a cell and its progeny accumulate mutations that give the cells a proliferative advantage over their normal neighbors, causing an abnormally large accumulation of the mutant cells, called a tumor. Is an abnormal cell division rate sufficient to explain the development of cancer?

7. It has been proposed that proliferating cells have cell-size checkpoints in their cell division cycle, where the cell can pause and assess whether it is large enough to proceed to the next step in the cycle, thereby ensuring that the cell divides at an appropriate size. One reason for thinking that proliferating cells must have such checkpoints is that the two daughter cells produced by a cell division are not always exactly the same size. If the bigger daughter cell were to grow faster than the smaller daughter cell, as intuitively one might expect, the size differences would be expected to get greater and greater over time, as the larger daughters would produce still larger daughters, which would then grow even faster. Yet, it is observed, for various proliferating cell populations in culture, that the distribution of cell sizes remains constant over weeks and months, suggesting that cells have cell-size checkpoints that allow the smaller daughter cells extra time to grow before they divide. But, we have seen that for aphidicolin-arrested Schwann cells, big cells grow no faster than small cells. If this were also the case for untreated, normally proliferating Schwann cells, and big and small proliferating Schwann cells were to grow and progress through the cell cycle at the same rate, would they need cell-size checkpoints to maintain a constant distribution of sizes as they proliferate? Explain your answer.

8. Because large yeast cells seem to grow faster than small yeast cells (at least when blocked in S phase), it has been proposed that proliferating yeast cells have cell-size

checkpoints that help them maintain their appropriate size. It has been shown that yeast cells divide at a size that is characteristic for a particular culture medium—in general, the more nutrient rich the medium, the larger the cell size at division. Moreover, when, switched from a nutrient-poor medium to a nutrient-rich medium, yeast cells rapidly adjust (within one division cycle) and start dividing at the larger size that is appropriate for the nutrient-rich medium. What does this latter finding imply about the nature of the postulated cell-size checkpoint mechanism?

9. When Schwann cells proliferate in serum-free medium containing optimal concentrations of IGF1 and GGF, their average size is less than half that of cells proliferating in medium containing FCS, without added IGF1 or GGF. The cells proliferate at roughly the same rate in the two conditions, and they maintain their characteristic cell size distributions over time. What do these findings imply about the FCS?

10. As mentioned in Question 8, when yeast cells are switched from a nutrient-poor medium to a nutrient-rich one, the cells rapidly adjust within one division cycle and start dividing at the larger size that is appropriate for the new medium. By contrast, however, when proliferating Schwann cells are switched from a serum-free medium containing IGF1 and GGF to a medium containing FCS, it takes about six divisions and about ten days before they acquire the larger average size of Schwann cells proliferating in FCS from the start. Can you explain why yeast cells and Schwann cells behave so differently in such switch experiments?

## 6. Answers to Discussion Questions

1. No. It is possible to make mice with extra copies of the GH gene, so that they make greatly increased amounts of GH. Although these mice grow to be about twice the size of normal mice, no matter how much extra GH they make, the mice do not even grow to be as large as rats, let alone humans. If one makes a mouse that is missing the receptors required for their cells to respond to GH (or the receptors required for their cells to respond to IGF1), the mouse grows to be about one third the size of a normal mouse, suggesting that the GH/IGF1 system is responsible for about two thirds of the growth of a mouse. Thus, while variations in the GH/IGF1 system probably account for some of the differences in size between individuals within a species, they are unlikely to account for the enormous size differences between species. We still don’t know why one species grows to be larger than another; the

differences are clearly determined by genes, but it is unclear which genes are the important ones.

2. Although we understand very little about the molecular basis of size differences between different cell types in the same animal, it seems unlikely that differences in concentrations of extracellular growth factors are mainly responsible. When rodent tissues are cultured in a plastic dish in fetal calf serum (FCS), for example, the different cell types in the tissue tend to maintain their differences in size, even though they are now exposed to the same concentrations of the mixture of growth factors contained in FCS. In exceptional cases, we know why a cell type becomes exceptionally large: skeletal muscle cells, for instance, are produced by the fusion of many muscle cell precursors (myoblasts), so that a mature muscle cell contains many nuclei; and, in general, the more complete sets of chromosomes a cell has the bigger it is. However, most cell types in our body contain the same number of chromosomes, and so differences in chromosome numbers cannot be the reason for most of the size differences. The differences in size presumably reflect differences in the genes expressed, but it is not known which genes determine the size differences.

3. Cell size at least partly depends on the total amount of protein a cell contains, so that, in general, large cells contain more protein than small cells. With double the amount of genes, a tetraploid cell would be expected to make twice as much mRNA, which encodes proteins, and twice as much ribosomal RNAs, which encode RNA components of the ribosomes, where proteins are made in cells. Therefore, one would expect a tetraploid cell to contain about twice as much protein as a diplod cell of the same type and therefore to be roughly twice the size.

4. Aphidicolin inhibits the enzyme DNA polymerase alpha, which catalyzes DNA synthesis in the nucleus. Thus, when cells in culture are treated with aphidicolin, nuclear DNA replication is blocked, and the cells arrest in S phase of the cell cycle and do not divide. Because the arrested cells can continue to grow, one can study cell growth, independent of cell-cycle progression. For example, one can test whether various signal proteins such as IGF1 and GGF stimulate cell growth, which can be followed over days; if the cells were proliferating, one would have to measure cell growth during a a single cycle, which typically is less than 24 hours.

5. The definitive answer is not known, but one possible explanation relates to differences in what limits the cell growth rate in the two situations. The mutant yeast

cells are growing as fast as they can in the presence of optimal nutrients (as they don't require signals from other cells), so that their growth is not limited by extracellular conditions; instead, their growth rate is limited by some factor or factors inside the cell, such as the number of ribosomes. Big yeast cells would be expected to contain more of these factors than small cells and therefore to grow faster. In the case of the aphidicolin-arrested Schwann cells, by contrast, we know that their growth rate is limited by the concentration of growth factors in the extracellular fluid: as one increases the concentration of IGF1 or FCS, the rate of cell growth increases. The mystery is how the arrested Schwann cells coordinate their protein synthesis with protein degradation (and secretion) to ensure that their growth rate is independent of their size.

6. No. If the mutant cells divided abnormally fast but did not grow abnormally fast, cell numbers would increase at an abnormally fast rate, but the cells would get progressively smaller, and there would be no increase in cancer cell mass. This probably explains why the drug rapamycin is effective against some forms of cancer. It inhibits a protein kinase called TOR (for target of rapamycin), which is a major driver of cell growth; TOR promotes cell growth by both stimulating protein synthesis and inhibiting protein degradation.

7. No, they would not. If large and small cells grow and progress through the cell cycle at the same rate, the progeny of large and small daughter cells would eventually return to the mean population cell size over time.

   Consider an unequal cell division that produces one daughter cell of mass 10 arbitrary units and one daughter of mass 1 unit. Assume that the subsequent divisions are all equal, producing daughter cells of exactly the same size. Following the first division, assume each daughter grows by 5.5 units. Thus, the smaller daughter would grow to 6.5 units before dividing to produce two daughters of about 3.2 units; the initial larger daughter would grow to 15.5 units before dividing to produce two daughters of about 7.8 units. In this way, as the cells continue to divide, the daughter cells and their progeny will tend to converge to a mean size of 5.5 units. The sizes converge because the larger cells do not double their mass in each cycle, and the smaller cells more than double their mass in each cycle. Thus, such cells will maintain a constant size distribution over time, even without cell-size checkpoints. It remains controversial, however, whether normal animal cells of the same type grow and divide at rates that are independent of their size.

8. At a cell-size checkpoint, a proliferating cell is thought to assess whether it is large enough to proceed to the next step in the cycle; if it is not, the cell pauses to allow more time for it to grow. It is not clear whether the cells assess their size, mass, or some other parameter that represents cell size. The finding that yeast cells can rapidly adjust the size at which they divide in response to an abrupt switch in nutrient conditions, suggests that the cell-size threshold that a cell has to reach at the postulated checkpoint must be rapidly adjusted to be appropriate for the new extracellular conditions. Because it is uncertain how cells assess their size at such checkpoints, so it is not known how the adjustment occurs.

9. First, it implies that the FCS contains both growth factors and mitogens for Schwann cells: if FCS contained mitogens but no growth factors, the cells would get progressively smaller with each division; if it contained growth factors but no mitogens, the cells would grow but not divide. Second, it implies that FCS contains growth factors that, together, are more potent at stimulating Schwann cell growth than IGF1, so that the cells grow more in each cell cycle and therefore divide at a larger size; the mitogen activity of FCS for Schwann cells, by contrast, is no greater than GGF.

10. As outlined in Question 8, proliferating yeast cells may need cell-size checkpoints to help them maintain their appropriate size, perhaps because big yeast cells grow faster than small yeast cells. Schwann cells may not need such cell-size checkpoints because big Schwann cells and small Schwann cells grow at the same rate, at least when arrested in S phase with aphidicolin. In fact, the behavior of Schwann cells in the switch experiment is pretty much what one would expect for cells proliferating without cell-size checkpoints: as outlined in Question 7, the progeny of large and small cells would eventually return to the mean population cell size over time, as long as large and small cells grow and progress through the cell cycle at the same rates. If Schwann cells do have cell-size checkpoints, they are clearly very different from those thought to operate in yeast cells. It seems that the size of a Schwann cell at cell division depends mainly on how fast the cell is growing and how fast it is going through the cell cycle, which, in turn, depend on the concentrations of the relevant growth factors and mitogens, respectively.

## 7. Explain or Teach These Concepts to a Friend

1. Explain the importance of cell growth in biology and cancer.
2. Explain what a cell-size checkpoint is.
3. Explain the respective ways that yeast cells and Schwann cells may use cell-size checkpoints or extracellular growth factors and mitogens to co-ordinate their growth with cell division.

## 8. Research the Literature on Your Own

1. What are the various ways one can measure cell size and cell growth?
2. What is the evidence for cell-size checkpoints in yeast cells?
3. What is the evidence for cell-size checkpoints in animal cells?
4. What intracellular signaling pathways regulate cell growth, as opposed to cell-cycle progression, in animal cells?
5. How does the TOR kinase promote cell growth?