Cell Growth

细 胞 生 长

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Cell Growth
CONTROL OF CELL SIZE
细胞生长

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Cover: Insulin receptors promote the growth of *Drosophila* fat body cells. Insulin receptors were overexpressed clonally, just in those cells with green membranes. Cells with red membranes are normal. Insulin-driven growth also increases the nuclear DNA (blue) in these highly polyploid cells. (Photo courtesy of Ling Li; for details, see J.S. Britton et al. [2002] *Dev. Cell* **2:** 239–249.)

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Preface

CELL GROWTH (INCREASE IN CELL MASS OR SIZE) is a highly regulated process, being subject to both temporal and spatial controls. It is usually coupled with cell division (increase in cell number) to give rise to an organ or organism of a characteristic size. In other cases, cell growth and cell division are unlinked; examples include oogenesis, muscle hypertrophy in response to an increased workload, and synapse strengthening during memory storage. Many of the molecules and mechanisms that control cell growth have been conserved in evolution from yeast to humans. Indeed, cell growth, along with cell division and cell death, is one of the most basic aspects of cell behavior. Furthermore, dysfunction of the signaling pathways controlling cell growth results in cells of altered size and can lead to developmental errors and contribute to a wide variety of pathological conditions, including cancer, diabetes, and inflammation. Considering its fundamental importance and clinical relevance, cell growth has not received as much attention as it deserves. We hope that this book will help redress this situation.

Creating this volume was an ambitious task, as cell growth is a broad and multifaceted subject. In 1957 (*Cancer Research* **17:** 727–757), M.M. Swann defined cell growth as "a not too precise shorthand word for those synthetic processes that do not appear, as yet, to be immediately connected with division and which provide the bulk of new protoplasm." To provide boundaries, while at the same time leaving some leeway, we asked the authors to provide their unique perspective on Swann's "processes." The first four chapters discuss cell growth in the context of development and/or cell division; Chapters 5 through 13 focus on individual molecules and mechanisms that control cell growth, and Chapters 14 through 20 describe cell growth in specific tissues.

We are extremely grateful to the authors for their contributions and enthusiasm, to Paul Nurse for writing a foreword while dealing with a trans-Atlantic move, and to our colleagues at Cold Spring Harbor

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Foreword

IN ADDITION TO BEING THE UNIT OF LIFE, the cell is also the unit of growth. Understanding what regulates the overall growth of a cell and how cell growth is coordinated with progression through the cell cycle are important problems in cell biology. In steady-state conditions, proliferating cells usually maintain a particular size, suggesting a strong link between cell growth and division, but how this is brought about remains unknown for most cells. Thirty years ago, these problems were at the forefront of cell-cycle research (see Murdoch Mitchison's classic book *The Biology of the Cell Cycle*, Cambridge University Press 1971) but, more recently, they have been relatively neglected, which is why the appearance of this volume on cell growth is so timely.

For steady state proliferating cells to maintain their size, the amount of an individual component and the size or mass of a cell must double in amount every cell cycle. Different patterns of growth during the cycle can achieve this. The simplest pattern is a step accumulation of the component or cell mass, with a doubling occurring over a restricted period of the cell cycle. A good example is the replication of a gene, which occurs during a limited part of S-phase. Such a pattern can apply to individual components but is unlikely to apply to the overall growth of a cell, as it would mean that all cellular components would be synthesized within the same restricted time period. In fact, a step pattern is deceptively simple, because a regulatory mechanism must be in place to ensure that synthesis stops once the amount of the component has doubled. When synthesis is determined by a template, as in the case of semi-conservative replication of a gene, monitoring such a doubling is straightforward. For other components, however, the regulatory mechanism must be able to monitor the absolute amount or concentration of the component during the period of synthesis so that this can be switched off once a doubling in amount has occurred.

Another pattern is where growth is linear with a rate doubling during a restricted period of the cell cycle. This occurs when the synthesis of a component or the overall growth of the cell is constant, but then doubles in a stepwise fashion during a limited period of the cycle. This is an interesting pattern because it suggests that some mechanism is limiting accumulation of the component or overall mass, and that this limitation is relaxed during the stepwise increase. An example of this would be when a gene is limiting for transcription; once the gene is replicated during S-phase, the presence of two templates doubles the rate of transcription. A linear pattern of overall cell growth implies that a single component, or a very restricted set of components, is limiting for growth; these limitations become relaxed at the rate-doubling point. In this situation, an increase in the levels of these limiting components leads to an immediate increase in growth rate. For free-living single-celled organisms such as yeasts, which are likely to have been under selective pressure to grow at a maximal growth rate, a limitation like this would probably be evolutionarily unstable. If only a single component is limiting, then it should be straightforward for the cell to evolve and make that limiting component more efficient, allowing an increase in growth rate. In multicellular organisms such as metazoans, selection for rapid growth may be unimportant, in which case certain components might well be limiting for overall cell growth. If this is the case, it should be possible to identify gene functions which, when more active, could drive growth more rapidly. The identification of such genes should indicate what limits overall growth rate in such cells. Given that cells in multicellular organisms are "social," communicating with other cells in the body, growth control may be regulated more by extracellular signaling mechanisms than by intracellular components. Another interesting aspect of linear growth followed by rate doubling is how the increase in rate of synthesis is controlled, because, again, some regulatory mechanism must operate to ensure that there is a step doubling in synthesis at the change point.

A third deceptively simple pattern is exponential growth, often favored by theoretical and more mathematically inclined biologists. An exponential pattern of growth means that the rate of increase of a component or overall cell mass is determined by the absolute amount of the component or cell mass at that time. As a cell gets gradually larger during the cell cycle, the rate of increase gets gradually larger, too. Such a pattern requires specific regulatory mechanisms to ensure an autocatalytic-like pattern of accumulation. It can be very difficult to distinguish experimentally between a linear with rate-doubling pattern and an exponential pattern, even though the explanations for these patterns are very different.

In exponential growth, the patterns of accumulation of different components must be coordinated so that they are all similar to the overall growth of the cell.

A second problem in cell growth control is how cell growth and division are coordinated to maintain cell-size homeostasis. The simplest explanation for cell-size homeostasis is that progression through the cell cycle is restrained by the need for the cell to attain a critical cell mass before certain cell-cycle events can be completed. Such controls have been demonstrated in yeast, where they have been shown to operate before the onset of S-phase and/or mitosis. If a cell is too small, it needs to grow more before carrying out these events. This is achieved by lengthening the cell cycle, thus restoring a normal cell mass. In contrast, if a cell is too large, it grows less, shortening the cell cycle, once again restoring a normal cell mass. The best evidence for critical-mass cell-cycle controls in eukaryotic cells are experiments in both budding and fission yeasts, which have shown that undersized and oversized cells largely return to a normal size within one or two cell cycles. However, evidence for such controls in metazoan cells is much less compelling (see Chapter 3).

Despite the fact that experiments establishing the existence of such critical-mass controls in regulating cell-cycle progression have been in place for many years, the nature of the molecular mechanisms that monitor cell mass remain unknown. Many models have been proposed, but working out which operate has been very difficult. One interesting feature of a major class of these models is the requirement to count absolute numbers of a component in the mass-monitoring network. This requirement comes about because there is often a concentration term in the equations used, with an absolute amount of the component divided by cell volume (where volume is assumed to be proportional to cell mass). In these models, measurements of a critical mass or volume require monitoring or generating a constant amount of a component. One component present in a constant amount per cell is the genome and molecules associated with it. Thus, a general class of cell-mass-monitoring mechanism could be imagined that involves titrating out a fixed number of sites associated with the genome. The genome is relevant here because the major events of the cell cycle controlled by the cell-mass controls are S-phase and mitosis, both of which involve the genome. This role of the genome is also consistent with the fact that bacterial, yeast, fungal, plant, and animal cells of increased ploidy have a proportionally increased cell mass, which could be a consequence of having more sites associated with the genome.

Another way to bring about cell-size homeostasis is for cell-cycle progression to be regulated by a timer or oscillator that measures an absolute

period of time from cell division to cell division, combined with a linear pattern of cell-mass increase. Time could be measured between the same events in succeeding cycles, such as S-phase or mitosis.

If the pattern of cell-mass accumulation is linear rather than exponential, cell-size homeostasis can be achieved without critical-mass controls, although it is inefficient. This comes about because, if growth is linear, with a rate that does not increase with cell size, then a small cell will accumulate mass faster per unit mass than a large cell. Thus, in a constant period of time between successive cycles, an initially small cell will grow proportionately more than an initially large cell. Over successive divisions, this will gradually shift smaller and larger cells back to the mean size of the population. This mechanism can work, but it is slow to achieve cell-size homeostasis, although to date there is little experimental evidence of support for this view (but see Conlon et al., Chapter 3).

This foreword has briefly touched upon some of the problems associated with cell growth and the cell cycle that were being discussed 30 years ago. Clearly, over this period partial answers have been provided to some of the issues described here. For example, we now know that cell growth is generally steady and continuous throughout most of the cell cycle. However, it is to be hoped that the renewed interest in these areas, as evidenced by the production of this volume, will allow some of these interesting issues to be resolved and extended. Not only does the mechanism by which cell growth is coupled to cell division need to be resolved, but also the regulation of overall cell growth in response to nutrients, growth factors, and other stimuli remains to be elucidated. Although the conversations occurred mostly long ago, it is a pleasure to acknowledge many fruitful discussions about these areas, particularly with Peter Fantes, Murdoch Mitchison, John Pringle, John Tyson, and Robert Brooks.

PAUL NURSE

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How Metazoans Reach Their Full Size: The Natural History of Bigness

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LIFE FORMS RANGE ENORMOUSLY IN SIZE, but as yet, we have little understanding of the mechanisms that determine the size of a cell or the size of an organism. Not only do we have little understanding of the mechanisms, but there is also a lack of appreciation of what is represented by growth control. Until recently, growth control was equated with the control of proliferation (Raff 1996; Su and O'Farrell 1998). However, the simple observations that cells can grow to different sizes, and that cells can divide without growth to produce larger numbers of smaller cells, suggest that the processes ought to be considered separately. It is recognized that growth should be considered in terms of increase in mass rather than increase in cell number. To many investigators, this appears to be a formalism, because they are so familiar with growth situations in which the two go hand in hand. The formalism becomes much more concrete when examining growth and cell proliferation in metazoans. It turns out that there is an extraordinary and fundamental segregation of organism growth and cell proliferation in the life histories of most metazoans.

There has been a recent surge of interest in growth control and wider recognition of the distinctions between the control of mass increase and the control of cell proliferation. Despite newly emphasized distinctions between growth and cell proliferation, in most of the systems that are currently being investigated, growth is largely exponential and cells are actively proliferating in close parallel. This includes proliferating yeast (Crespo and Hall 2002), expanding tissue culture populations, and the

multiplying cells of the *Drosophila* imaginal disc (Neufeld et al. 1998). These systems provide experimental access to numerous questions about the mechanisms that couple cell-cycle progression to increase in cell mass and the interactions that coordinate growth with nutritional conditions. Furthermore, since proliferative growth marks the lifestyles of the unicellular predecessors of metazoans, this mode of growth is presumably primordial and fundamental. However, if one looks beyond these systems and examines how growth is integrated into the life cycles of different metazoan organisms, one finds that growth often does not parallel cell proliferation. Indeed, they are often completely out of synchrony, such that growth and cell proliferation occur in different parts of the life cycle. I describe the major metazoan growth programs and the integration of growth into the life cycles of different organisms. It will be seen that the separation of growth and cell proliferation are early adaptations in metazoan evolution. To understand the origin and purpose of these features of growth regulation, I have built a discussion based on the fact that the evolutionary history of metazoans influences present-day regulatory programs.

The considerations introduced in this chapter also promote a broader recognition of the importance of growth control in the natural history of organisms. Growth control goes beyond controlling the size of cells or the size of an organism. It is growth control that properly proportions different body parts and consequently shapes the body. My interest in growth (size) control was first piqued by its astonishing precision—a precision that is illustrated by near perfection of bilateral symmetry. This interest was fueled and sustained by recognition of the huge contributions that growth control makes to development and evolution. Each and every part of a body is sized appropriately, and evolutionary adaptations—the specialized finger of the aye-aye or the nose/trunk of an elephant--involve extraordinary modifications in the relative sizes of different body parts. To appreciate all these issues, it is important to examine how metazoans grow.

I begin this chapter with an outline of what I call biological constraints—factors that affect every metazoan to create "problems" that must be solved or evaded in the development and growth of every organism. It appears that a few of these constraints limited, and hence directed, the evolutionary history of current growth paradigms. Among other things, these constraints favor a separation in which growth and cell proliferation occur at different stages of an organism's life cycle. Indeed, consideration of when and where growth occurs and when it is limited will lead us to identify several distinct programs of growth control.

CONSTRAINTS ON BIOLOGY AND GROWTH

Conservation of Mass

Even evolution cannot evade the dictates of the first law of thermodynamics. Consequently, an organism without a mouth or alternative means of nutrient uptake cannot grow (increase in mass). Although this may sound trite, it has an enormous impact. Eggs generally come with protective shells to isolate them from the harsh environments in which they are laid. Without input of new mass, the hatchling can be no larger than the egg that was laid. Indeed, since the vast majority of metazoan embryos have no means of nutrient uptake, they must develop at least until they are competent to feed before growth can commence. Feeding usually requires at least a mouth and an alimentary canal, structures that can only be produced following significant development. Thus, this constraint predicts that extensive cell proliferation and tremendous steps in development precede growth. Indeed, as discussed below, in the life cycles of many organisms, cell proliferation precedes growth, and it is likely that this separation was a feature of primitive programs for metazoan growth.

Mitosis Is Incompatible with Many Differentiated Structures and with Many Steps in Morphogenesis

Mitosis is remarkable, not only because of what it accomplishes, but also because of the way in which it appropriates or disrupts much of the cellular machinery in order to achieve its ends. The microtubular cytoskeleton is remodeled from its interphase arrangement to assemble the mitotic spindle. The actin cytoskeleton is rearranged to create a cytokinesis furrow and to drive its invagination through the cell. The Golgi is disassembled, transcription arrested, translation suppressed, and cellular processes withdrawn. These demands of mitosis would wreak havoc with the structural specializations of many differentiated cells. It is obvious that syncytial muscle cells cannot replicate mitotically. Similarly, the specializations of neurons, or the crystal cells composing the lens of the eye, or the Schwann cells that encase the axon in myelin are not compatible with mitotic proliferation. Although mitosis may successfully duplicate less dramatically differentiated cells, it remains a disruptive process. For example, even though mitotic epithelial cells retain connections to the rest of the epithelium, mitosis nonetheless locally compromises the ability of the endothelium to prevent leakage of large molecules from the vasculature (Lin et al. 1988; Baker and Garrod 1993). Consequently, mitosis tends to interfere with the function of terminally

differentiated tissues. In the embryos of many organisms, this difficulty does not arise because proliferation is largely limited to the early stages of development prior to differentiation.

Distance-dependent Mechanisms

Some phenomena, such as the transport of oxygen by diffusion, are distance dependent. It is easy to recognize that large body sizes are associated with structures and mechanisms designed to transport oxygen and eliminate waste. Unfortunately, it is perhaps also easy to overlook the fact that, during evolution, body size was constrained by the ability to transport oxygen and eliminate waste.

This constraint is not limited to the difficulty of getting things in and out of a large organism. Some biological mechanisms, most notably those that pattern an embryo, are founded on distance-dependent phenomena. One example is the conserved mechanism involving a secreted *dpp*/TGF-β signal from one side of an embryo interacting with an inhibitory signal (*short gastrulation*/Chordin) from the other side to create a morphogenetic gradient that patterns the dorsal/ventral axis (Ferguson 1996). Hence, patterning works best at particular scales, and the evolutionary conservation of the mechanisms that pattern the body axis might demand conservation of size at crucial stages in the patterning process.

PROGRAMS OF GROWTH IN METAZOANS

The Big Egg Paradigm

Although there is a natural tendency to focus on our own biology, uterine support of embryonic growth is extraordinarily rare (about 4,000 eutherian species out of about 1,000,000 total metazoan species). In most extant species, including the evolutionary predecessors of uterine mammals, a mother must pack into the egg sufficient reserves to carry development far enough to produce a feeding animal, and no growth can occur until feeding starts. To achieve this end, eggs are big—sometimes very big. Frog eggs are about 10^5 times larger than a conventional somatic cell. Let us consider the significance of the production of a large egg in terms of the total growth of an organism during its life cycle.

Caenorhabditis elegans provides a simple example of metazoan growth issues. A germ-line stem "cell" grows extremely rapidly in a syncytial gonad to about 1000 times its starting size (Fig. 1). Cell proliferation is largely confined to the early part of embryogenesis and occurs within a

closed egg shell without any growth. Morphogenesis then ensues, again without growth, to produce (at hatching) a small L1 juvenile. The worm then feeds and grows about 100-fold to produce the adult worm. This growth is almost exclusively by cell enlargement, as there is little postembryonic cell division other than cell proliferation associated with the development of the reproductive organs.

Several features of this description are notable. First, at least in terms of exponential growth or fold increase, the growth that produces the oocyte makes a contribution that is comparable to or larger than the growth during zygotic life. Second, there is an almost complete separation of growth, cell proliferation, and differentiation/morphogenesis. Third, growth can be divided into two phases, one prior to proliferation (and prior to fertilization), in which the growth of the oocyte is supported by the mother, and one after the period of proliferation, which is supported by the feeding of the hatchling.

The *C. elegans* program, with its sequence of growth, proliferation, and morphogenesis, followed by a second period of growth, satisfies the constraints that I described above. The egg is large enough to produce the 550 cells of the hatchling worm without growth, and, by limiting the vast majority of the cell division to the early stages of embryogenesis, the program largely avoids the difficulty of dividing differentiated cells. The early developmental paradigm of production of a large egg, followed by rapid division and subsequent differentiation, has been largely conserved, even if its features are masked in eutherian mammals.

C. elegans is very small. How are larger organisms produced? It appears that larger size is achieved by adding later stages of growth. Below, I describe a stage of development that is conserved between diverse organisms. This so-called "phylotypic stage" will serve as a useful reference point for comparison of the growth programs of various organisms, small and large, and for identification of the stage of development at which evolution has introduced variations in size.

A Universal Embryonic Size

As noted by von Baer and emphasized by Haeckel during the early years of the incipient field of developmental biology, embryos of very different species look remarkably similar at the stage following gastrulation, just after the body axis is morphologically established. The similarity applies to size as well as to morphology. Whether an embryo will produce a mouse or a whale, a minnow or a tuna, the postgastrulation embryos are of very similar size. Indeed, embryos of organisms as diverse as leech,

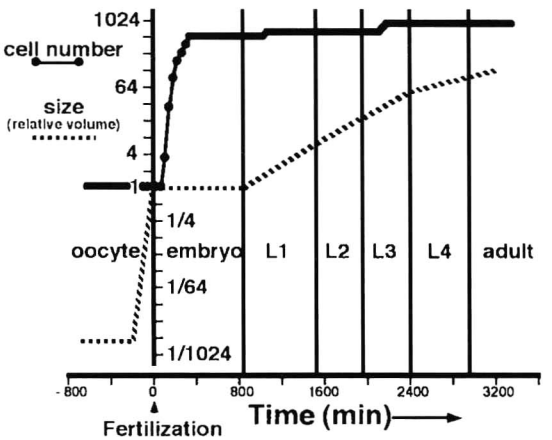

Figure 1. Growth and cell proliferation occur at different and largely nonoverlapping stages in the life cycle of the nematode *C. elegans*. Time relative to fertilization is indicated along the bottom, and the vertical axis, a logarithmic scale divided in intervals of a factor of two, indicates both cell number and size relative to the size of an egg. Expansion of somatic cell number (*solid line with filled circles*) by cell proliferation occurs almost exclusively during the first third of embryogenesis. This proliferative phase precedes much of the morphogenesis and differentiation, which occur during the later two-thirds of embryogenesis. Because differentiation and morphogenesis are segregated to a later stage, they avoid the potentially disruptive influence of mitosis (see text). Growth (*segmented line*) is also dramatically separated from cell proliferation. There is no growth during embryogenesis, which occurs within an enclosed environment inside the egg shell. Growth awaits hatching and the onset of feeding of the juvenile worm. Because this growth phase occurs after the major cell proliferative phase, growth depends largely on expansion of cell volume. In addition to this growth, the mother sponsors embryonic development via a specialized form of growth in which the oocyte grows tremendously. The germ-line portion of the ovary is syncytial, so that numerous nuclei make a communal contribution to the production of additional syncytial cytoplasm (growth) that supports the production of oocytes. It is difficult to plot this growth. Instead, I have plotted the extraordinarily rapid expansion of incipient oocytes in the distal part of the linearly arranged ovary. Beyond mitotic and meiotic zones, the syncytial cytoplasm is divided into modestly sized "cells," as membrane surrounds a single nucleus and an allotment of syncytial cytoplasm. The newly formed "cell" is only incompletely cellularized, as it maintains a cytoplasmic connection to the syncytium. A flow of cytoplasm to the incipient oocyte drives its very rapid growth, as indicated in the graph.

Drosophila, fish, frog, and mouse are all about 1 mm long when they first elongate along the anterior–posterior body axis. Although size constancy is not precise, and the *C. elegans* embryo is substantially shorter (~100

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