

# Cell Size Control

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Growth in biological systems is defined as the accumulation of mass, which leads to an increase in size. In this article, we discuss how cells, organs, and organisms normally control growth, and how deregulated growth can lead to a variety of pathological conditions.

## Introduction

### Controlling growth in cells organs and organisms

A major unanswered question in biology is – how do living organisms control their size? There are dramatic differences in the size of animals; for instance, blue whales, the largest animal that has ever lived, can grow up to 110 ft in length and weigh over 360 000 pounds, while the fairyfly, the smallest insect, is only 0.01 in. in length. Even within a species, there are variations in size. Consider humans, our average heights and weights change as we grow, and are also different between age groups, race and gender. Although there is such variation among organisms, the organs and appendages of each individual are normally in proportion with the body as a whole. What controls the size of organisms and their components? For the most part, the answer lies in the genes. Genetic variations control the size to which organs or organisms can grow. Genes control the synthesis and transport of hormones and growth factors, which act on different tissues to control overall body growth. However, environmental factors are also important. A good laboratory example is the developing fruitfly, which when fed a nutrient-restricted diet, develops normally, but is significantly smaller than flies fed a normal diet. This can also be seen in humans where malnourished children develop a growth deficiency. Thus, factors such as nutrition impinge on the genetic programme to control animal size.

The overall size of any animal or organ depends on the number and size of its cells. The average animal cell size is approximately 10–20  $\mu\text{m}$  in diameter. Such small size results in a large surface area-to-volume ratio that allows for efficient transport of materials in and out of cells. If cells were too large, transport would be inefficient and incapable of supporting the metabolic demands of the cell. Thus, large organisms, like elephants, are made of more cells, not bigger cells. Within our bodies, there are 20–30 trillion cells, which make up approximately 200 different varieties. While the average cell size is small, the range of cell sizes is diverse (Figure 1). The human oocyte grows up to 100  $\mu\text{m}$  in diameter. Neurons, like Purkinje cells of the brain and motor neurons can grow much larger than the average cell. Some Schwann cells, which are companion cells for nerve cells,

are 30–60  $\mu\text{m}$  in embryonic life and grow coordinately with the growing axon during development, potentially reaching up to 1 mm in adult life. Size variation can be found in cells circulating in the blood; for instance, neutrophils are  $\sim 10 \mu\text{m}$  in diameter while macrophages are 20–30  $\mu\text{m}$  in diameter. These variations in cell size raise a fundamental biological question: Do cells regulate their growth?

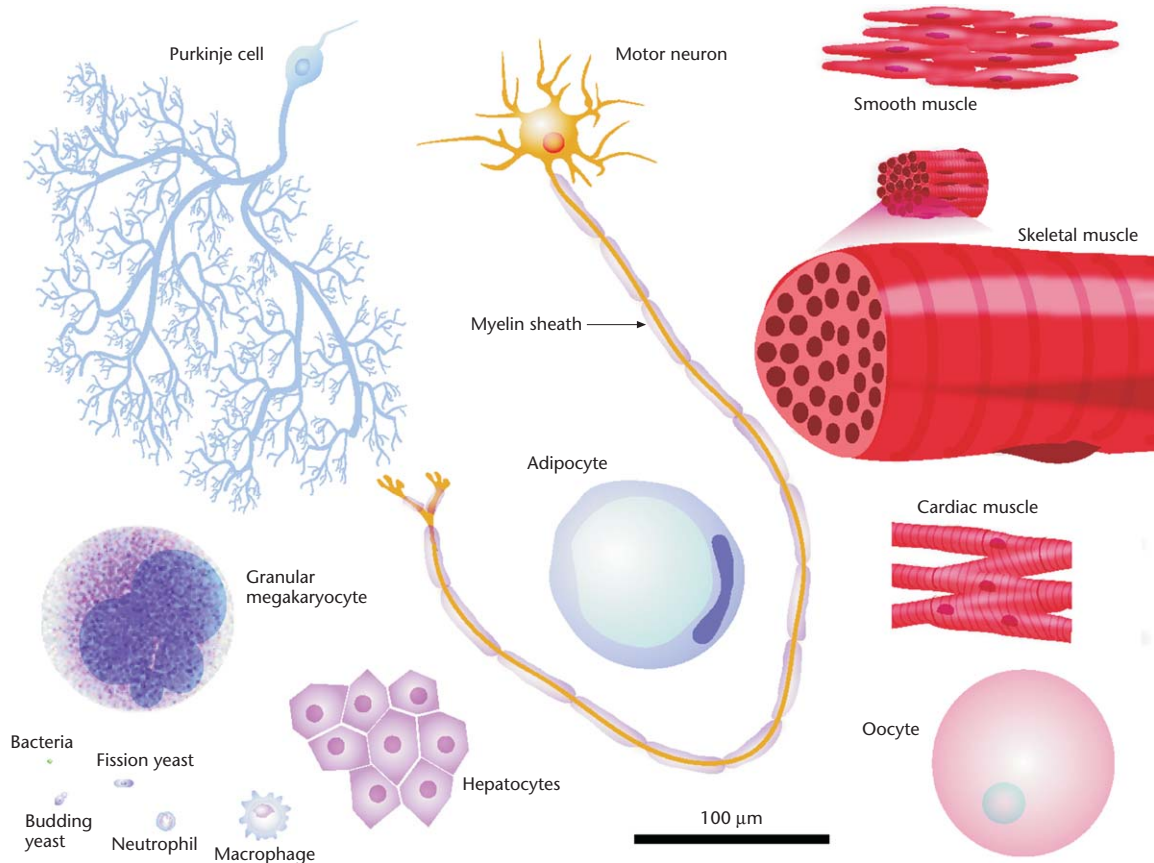
Cell growth is the process of building mass to increase size, not to be confused with cell division, the process whereby one cell divides into two. All cells grow to a final size, and in every passage through the division cycle, cells must grow to an optimal size prior to dividing. How do cells increase their mass? Cell size can be proportional to deoxyribonucleic acid (DNA) content, thus increased DNA content or ploidy is associated with some large cells. Granular megakaryocytes, the platelet-producing cells of the bone marrow, are one example. Megakaryoblasts are about 20–30  $\mu\text{m}$  in diameter. These cells mature into promegakaryocytes, which develop into granular megakaryocytes that are  $\sim 100 \mu\text{m}$  in diameter. Characteristically, these cells do not divide, but undergo multiple rounds of DNA replication in the absence of division, generating a total DNA content ranging from 8c to 64c, with 1c equal to the haploid DNA content. This process called endoreplication is a widespread strategy for increasing cell size found in protists, plants and animals. It remains to be determined whether increasing genome size increases the amount of material in a cell, or whether cells increase genome size to cope with the demands for increased growth. Fat cells or adipocytes can reach 85–120  $\mu\text{m}$  in diameter, but for these cells, increased size is due to intracellular accumulation of lipids. Another way cells increase mass is by actively synthesizing more total biomass without increasing DNA content or accumulating lipids. Terminally differentiated neurons, cardiac muscle and multinucleated adult skeletal muscle cells increase mass this way, as well as most dividing cells prior to division.

## Introductory article

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**Figure 1** Biological variations in cell size. Eukaryotic cells average 10–20  $\mu\text{m}$  in diameter, but the range to which cells grow is diverse. The cells in this figure are drawn close to scale to emphasize the size variations. A scale bar is drawn for comparison. Cell size is determined by a variety of processes. For granular megakaryocytes, large cell size is associated with multiple copies of the genome (polyploidy). Fat cells are large because they accumulate excess lipid. Other cells contain varying amounts of total biomass, mostly protein, while maintaining a diploid genome size (haploid in oocytes). Some of the largest cells in the body are neurons like Purkinje cells and motor neurons, which grow by increasing cell mass. Likewise, adult polyploidy skeletal muscle cells, depending on the muscle type, can grow to 10–100  $\mu\text{m}$  in diameter by increasing cell mass.

Since the size of an organism begins with its cells, this article will begin by discussing how cells control growth. We will focus on mechanisms that control cell mass accumulation without increasing DNA content as the biology behind this type of growth has a widespread role in human physiology and disease. In the second part, we will discuss the complicated relationship between animal cell growth and organ and organism growth both in normal physiology and in disease. Finally, we will draw a comparison between cell growth in animals and plants, which increase in size by a very different mechanism.

## Increasing Cell Mass by Building New Protein

By definition, cell growth is the accumulation of mass, which results in increased cell size. Cells generally grow

during every division cycle so that daughter cells are not reduced in size compared to the mother cell. Moreover, cells that stop dividing, like neurons and skeletal muscle, continue to grow to reach optimal size. The major building blocks of a cell can be placed into four categories of organic molecules: nucleotides, amino acids, sugars and fatty acids. These are assembled into higher-order molecules or macromolecules; nucleotides into nucleic acids (DNA and ribonucleic acid (RNA)), amino acids into proteins, sugars into polysaccharides and fatty acids into fats, lipids and membranes. While water makes up roughly 70% of total cell weight, macromolecules make up nearly the rest, constituting around 25%, with ions and small molecules making up the difference. By far, proteins constitute the most abundant macromolecules in animal cells, making up 18% of total mammalian cell weight. (Plant cells contain a considerably larger amount of carbohydrates such as starch, glycogen and cellulose, which are important for energy, storage and structure – discussed below). In addition to

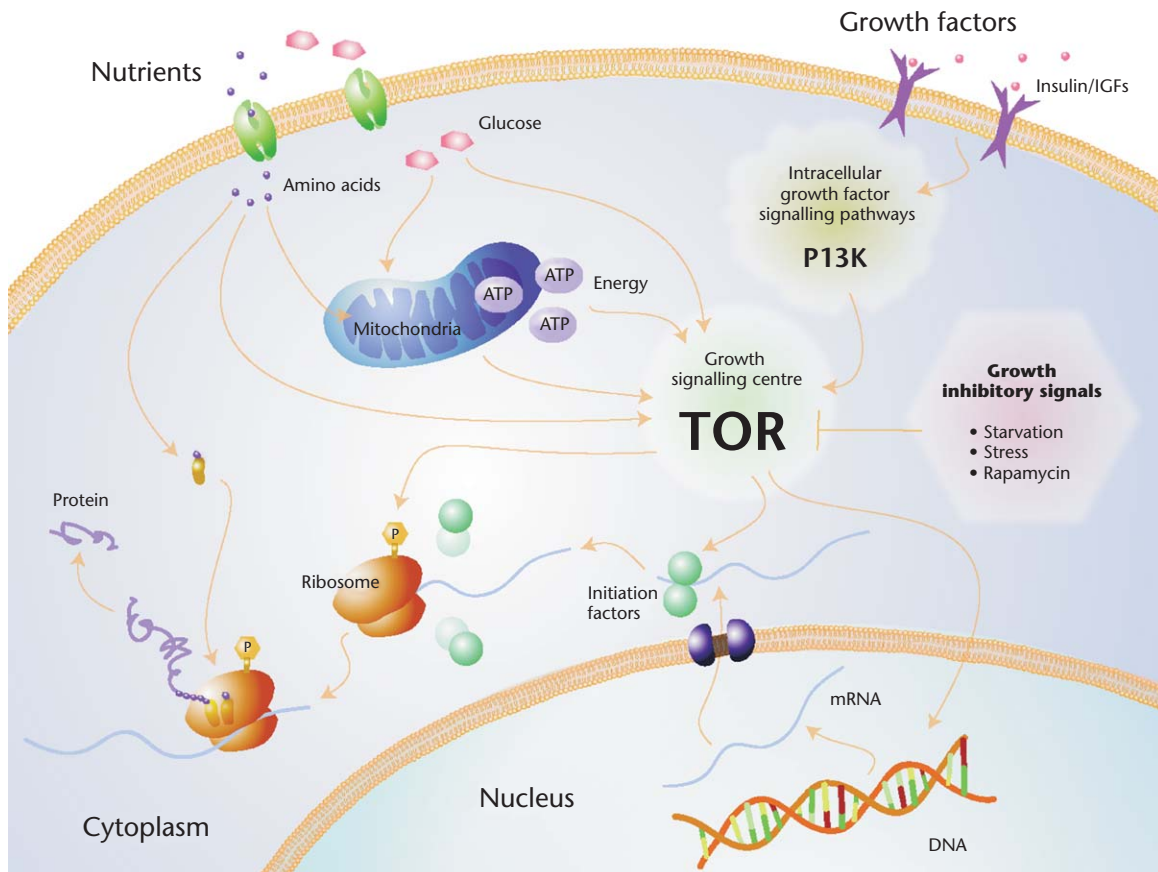
being a potential energy source, proteins perform all the important functions of the cell, serving as enzymes, structural components, channels, pumps, motors, signalling messengers, growth factors, hormones, antibodies and others. Regulating synthesis of protein is a major step in increasing cellular biomass and it depends on the availability of the protein synthesis machinery and the presence of intra- and extracellular growth signals (**Figure 2**).

Proteins are synthesized in the cytoplasm by the ribosome, a huge macromolecular machine. An ordinary eukaryotic cell has millions of ribosomes capable of adding two amino acids to a polypeptide chain per second.

In eukaryotes, protein synthesis begins with an initiation step, where messenger ribonucleic acids (mRNAs) to be decoded associate with ribosomes. Initiating protein synthesis is controlled by cytoplasmic regulatory factors called

'initiation factors', which regulate association between the ribosome and mRNA. During growth, activation of initiation factors is controlled by phosphorylation as a result of signals from extracellular sources, such as growth factors. Growth factors are small peptides that bind receptors on the extracellular surface of cells and activate intracellular signalling pathways. Insulin or insulin-like growth factors (IGFs) are examples of important growth factors. When insulin or IGFs bind their receptors (the insulin receptor) on the cell surface, an intracellular signalling pathway is activated triggering the phosphorylation of initiation factors.

In addition to growth factors, nutrient availability and energy regulates protein synthesis. Making new protein is energetically very costly, so it makes sense that protein synthesis is coregulated by metabolic pathways. Amino



**Figure 2** Growth signalling in eukaryotic cells. Within eukaryotic cells is an integration centre where intra- and extracellular growth signals converge. The central regulator of this centre is a protein called TOR. TOR senses the incoming signals and relays the growth message to the machinery that builds new protein. Since protein constitutes the majority of the biomass of a cell, building new protein is a major way that cells increase their size. Inputs to TOR are generated by nutrients such as amino acids, which serve as both building blocks for making protein and as signals that activate TOR, and glucose, which is the fuel for making cellular energy in the mitochondria. Signals directly from the mitochondria and ATP may also signal to TOR. Extracellular signals from growth factors, such as insulin and IGFs also control TOR by activating intracellular signalling pathways like the P13K pathway. Once activated, TOR triggers protein synthesis by activating initiating factors, which catalyse the association of mRNAs with the ribosome, and by activating the ribosome directly. TOR also turns on genes that are important for making new protein. Starvation for nutrients, stress or the antigrowth drug rapamycin can block protein synthesis by inhibiting TOR.



acids are particularly important, not only as the building blocks of proteins, but like growth factors, the presence of amino acids can promote phosphorylation and activation of initiation factors. Other nutrients, like glucose, are also important. Glucose serves as the main fuel for generating cellular energy in the form of adenosine triphosphate (ATP).

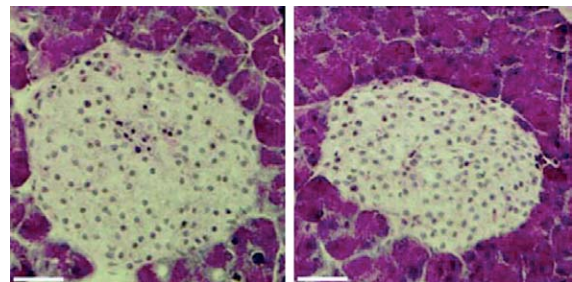
Since growth factors and nutrients are important for activating protein synthesis, it seems likely that cells have mechanisms for integrating these diverse inputs. In fact, models have recently emerged predicting the existence of a 'growth-signalling network'. The network is composed of many proteins collectively functioning as an integrating centre, receiving inputs from growth factors and nutrients and relaying a growth signal to the protein synthesis machinery. The network is an ancient mechanism of controlling cell growth as it has been conserved during evolution. The defining component and central regulator of this network is a large protein called TOR (target of rapamycin), which activates the initiation factors. In single-celled organisms, like yeast, TOR is regulated solely by nutrient availability in the surrounding environment. The most important nutrients are amino acids, but secondary signals from glucose metabolism and the mitochondria, the centre for cellular energy production, are also important. In multicellular organisms, where cells exist in close communities in a closed system, regulation of TOR is under dual control by nutrients and growth factors. Growth factors relay to cells the state of the surrounding environment. During tissue growth, for instance, the body makes growth factors that coordinately regulate growth of similar cells in tissues so that the proper developmental plan is implemented. TOR senses if sufficient nutrients are present to build protein, and whether growth factors are signalling the 'ok' to activate the machinery; if conditions permit growth, TOR will relay the signal to start building mass.

During periods of increased growth, cells also increase their ribosome content in a process called ribosome biogenesis. Having more ribosomes helps growing cells cope with the demand for making new protein, and ribosomes themselves can account for up to 40% of the total cellular mass. Making more ribosomes involves the expression of genes required for ribosome biogenesis and translation of specific preexisting pools of mRNAs that encode additional ribosome biogenesis factors. Like activation of initiation factors, ribosome biogenesis is controlled by extracellular growth factors and nutrient availability through TOR. Activating TOR leads to phosphorylation of the ribosome, as well as to expression of genes whose products function in making new ribosomes. Phosphorylation directs the ribosome to translate the specific pools of mRNAs encoding ribosome biogenesis factors first, before allowing translation of other mRNAs. By prioritizing synthesis of these mRNAs and activating ribosome biogenesis genes, cells ensure that

enough ribosomes are generated before bulk protein synthesis begins.

TOR is named after a naturally occurring chemical called rapamycin that binds to TOR and inhibits its function. Because it inhibits TOR, rapamycin inhibits cell growth, and thus has become an important clinical drug. Rapamycin is currently approved as an immunosuppressant in organ transplants as it can suppress growth of immune cells that attack foreign tissues. Rapamycin is also used to coat coronary stents used in balloon angioplasty. The antigrowth properties of rapamycin block restenosis (the narrowing or reblockage of an artery) at the site of the stent by reducing the growth of scar tissue, a complication often associated with angioplasty. The growth inhibitory properties of rapamycin have also raised interest in the drug as an anticancer agent, propelling it into ongoing clinical trials.

Once sufficient size is attained, cells cease growth, and in the case of dividing cells, start the division process. Removal of growth factors stops growth, as can the presence of proliferation signals called mitogens. Growth also stops if conditions become unfavourable for growth, such as by starvation for nutrients or oxygen. In a clinical condition, prolonged starvation for protein can result in a reduced mass of insulin-producing pancreatic  $\beta$ -cells predisposing individuals to glucose intolerance. This condition, called protein malnutrition diabetes, can be mimicked in laboratory mice by partially inactivating the growth network (Figure 3). Cells also can reverse growth by actively degrading proteins in a process called autophagy. Autophagy is important for degrading and recycling old proteins during starvation, but is also important in normal differentiation and development. Thus, growth can be thought of as a balance between opposing forces that synthesize and degrade protein.



**Figure 3** Starvation can cause diabetes by reducing the mass of insulin-producing cells. Protein malnutrition diabetes is a condition caused by reduction in mass of the insulin producing  $\beta$ -cells of the pancreas as a result of starvation. In laboratory mice, partial inactivation of the growth network by genetically inactivating one of the downstream effectors of TOR mimics the effects of protein starvation. These mutant mice have reduced levels of insulin. Consistent with low levels of insulin, pancreatic sections showing  $\beta$ -cells from the islets of mutant mice are decreased in mass (right) compared to islets of normal mice (left). This condition is one example of the importance of maintaining proper cell size. Pancreas sections stained with haematoxylin and eosin. Bar, 50  $\mu$ m. From Pende M *et al.* (2000).

## Human Diseases of Cell Growth

Tuberous sclerosis (TSC) and the related disease lymphangioleiomyomatosis (LAM) are diseases of abnormal cell growth. In the case of TSC, 1–2 million people worldwide are affected, developing benign tumours in vital organs such as the brain, kidney, lungs and skin, causing severe complications in many cases. Some brain tumours of TSC patients contain giant cells that block the normal flow of brain fluids. TSC is caused by a mutation in either of the two genes, TSC1 or TSC2. The products of these genes function together to restrain TOR activity. Without TSC1/TSC2 function, growth signalling is too strong and results in formation of the characteristic benign tumours. Another disease, Peutz–Jeghers syndrome (PJS) is also characterized by benign tumours in the stomach, intestine and skin. PJS is linked to a mutation in the *LKB1* gene, which normally functions to restrict growth in response to low energy.

As discussed, the insulin-signalling pathway is important for growth. The main messenger that propagates the intracellular response to insulin or insulin-like growth factors is a protein called PI3 K. While the function of PI3 K is to activate other intracellular molecules required for growth, another molecule, phosphatase and tensin homolog deleted on chromosome 10 (PTEN), reverses its function to balance the level of signalling. Thus, loss of PTEN function results in overactive insulin signalling. Mutations in PTEN are linked to a variety of abnormal growth syndromes including Cowden syndrome (CS), Bannayan–Riley–Ruvalcaba syndrome (BRRS) and Proteus syndrome (PS, the so-called elephant man syndrome). Patients with PTEN mutations also have a high risk for cancer.

Is there a prevalent role for the growth network in cancer? TOR, being an integrating centre for diverse signals, and relaying the signal to a variety of outputs, is an essential gene in all eukaryotic organisms examined. Deregulation and hyperactivation of many upstream TOR signals are linked to cancer, emphasizing the potential of TOR inhibitors as anticancer agents. Moreover, deregulation of many downstream TOR targets are linked to cancer, reinforcing the importance of this network in the disease. Curiously, no mutations in TOR itself are linked to cancer, perhaps reflecting the critical role of TOR in cell viability, or, more likely, underlying the fact that relatively little is known about its mechanism of function in the cell. Targeting TOR and other TOR network genes with inhibitory drugs is a focus of many anticancer strategies.

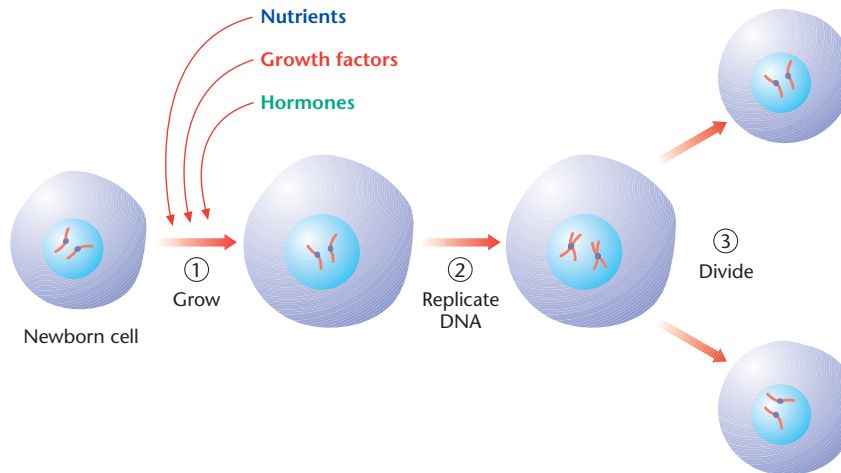
## Coordinating Cell Growth and Cell Division

Cell growth and cell division must be coordinated during the life cycle of a eukaryotic cell. Generally, during the cell

cycle growth phase, cells double in size so that after division, average cellular size is maintained. Lack of growth regulation would lead to heterogeneity in size. However, in some cases cells grow without dividing, while other cells can divide without growing. An interesting example is the human egg, which undergoes a large growth phase increasing in biomass in the absence of division. Once fertilized, the egg will then initiate multiple rounds of division in the absence of growth. In the 1970s, pioneering studies using the unicellular organism yeast uncovered many genes important for controlling passage through the cell life cycle. With the revolution in molecular biology well underway at the time, identification and characterization of these genes revealed that the mechanisms controlling cell division had been remarkably conserved during evolution. For nearly the next 30 years, biology was inundated with thousands of magnificent discoveries in cell division control, leading to amazing progress in understanding cellular physiology in normal cell division and disease like cancer. However, insight into the question of what controls cell growth lagged behind. A likely reason is growth control in single-celled organisms like yeast cells is only partially conserved with cells in multicellular organisms. In retrospect, this is not surprising as yeast growth is directly regulated by environmental nutrients. Cells in multicellular organisms, while partly regulated by nutrients, additionally rely on extracellular growth factors. Proliferating animal cells also require mitogens. Mitogens are like growth factors, but function to activate intracellular pathways, which license cells to divide (replicate DNA and initiate mitosis). Exactly how growth and division are coordinated is still a mystery.

When do cells grow during the cell cycle? In order to maintain constant average size during division, cells must double their mass before dividing otherwise they would get smaller with every division. To allow this, intervening ‘gap’ or G-phases exist that allow cells to accumulate mass prior to physically dividing. For most cells, it is the G<sub>1</sub>-phase, which is most critical to the growth-control programme (Figure 4). During G<sub>1</sub>, cells sense nutrient availability and the presence of extracellular signals and determine whether conditions are favourable to grow. If the combined signals indicate stop growing, then cells may enter a resting or G zero (G<sub>0</sub>) state in which they may become specialized or remain at rest until stimulated to grow in the future, or in some cases they die in response to pro-death signals, a process called apoptosis. If the combination of nutrient availability and extracellular signals indicate grow, then cells will increase in mass until the appropriate size is attained and subsequently initiate S-phase, irreversibly committing to at least one round of division.

In the fission yeast (*Schizosaccharomyces pombe*) and budding yeast (*Saccharomyces cerevisiae*), cells seem to have internal systems in place to monitor growth and coordinate it with division, only committing to division once a critical mass is attained. For budding yeast, like animal



**Figure 4** Cells must grow during every passage through the cell cycle. Newborn cells must grow in size prior to dividing so that cell size does not progressively decrease with each division. Cells generally grow in the G1-phase of the cell cycle by increasing the rate of protein synthesis. G1 growth is influenced by nutrients, growth factors and hormones. Once the appropriate cell size is reached, and signals are present that permit cell cycle progression, growth slows down and DNA synthesis begins in S-phase. Once the genome is duplicated and cells prepare for division, the full-sized mother cell will divide into two smaller daughter cells and the process is repeated. In yeast, intracellular mechanisms monitor size in G1, restraining S-phase until a critical size is attained. It is not known how animal cells maintain size control as evidence both supports and contradicts the existence of a size-monitoring mechanism.

cells, growth occurs mostly in G1 and it is predicted that a 'size sensor' determines whether cells are large enough to commit to cell division. Yeast also divide at a smaller size in nutrient-poor conditions, indicating the size-control mechanism is influenced by nutrient availability. Candidate mechanisms have been identified that may function as size sensors and the challenge now is to determine if similar mechanisms function in animal cells. An interesting question is whether animal cells have mechanisms in place to slow or inhibit growth in nutrient-poor conditions. This is particularly interesting to cancer biology as cancer cells may lose sensitivity to nutrient deprivation, allowing them to grow and proliferate in the harsh nutrient-limiting environment of a tumour.

## Organ and Organism Growth in Humans

The various organs in our bodies assume a variety of different sizes. Similar to cell growth, organ growth is the process of increasing the size or mass of an organ. What is the relationship between cell growth and organ growth, and to the growth of an entire organism? **Most developing organs increase mass by increasing cell number, a coordinated effort of growth and division in individual cells. But during adult life, cells from many tissues lose the ability to divide and are only capable of increasing mass by increasing the cell size.** Thus, the relationship between cell growth and organ or organism growth is complex and varies in different tissues or developmental stage. There are many interesting growth characteristics of organs and organisms between different species, and understanding the relationships between the growth-control programmes is an active

area of research. In the following section, we will focus on how human tissues increase mass during development and adult life, and to what extent cell growth and cell proliferation are involved. Where appropriate, we will review human conditions or diseases that result from defects in organ and organism growth control.

How do tissues grow during development? There is no one unifying strategy that tissues use to build mass. For instance, **tissues can increase mass or size by increasing the number of cells in a tissue, by increasing the size of cells in a tissue, by increasing the amount of intercellular material, which is the case in bone or cartilage or by a combination of these methods.** During rapid embryonic development, tissue progenitor cells become specialized by the process of differentiation. At the same time, tissues are also being shaped in the process of morphogenesis. The complex growth scheme of coordinated differentiation and morphogenesis involves a combination of cell growth, cell division and cell death. **Cell death, or apoptosis, is the mechanism by which a single cell, through a specific programme, self terminates. Apoptosis can be triggered internally by a genetic-programme, or it can be regulated by external factors. The importance of apoptosis during growth is exemplified in determining the final shape of our fingers and the roofs of our mouths, and in development of the neural tube and certain reproductive structures. During fetal development, balance between cell growth and proliferation – which increases cell number, and apoptosis – which decreases cell number, determines both the rate of growth and final mass of a tissue.**

Growth in postnatal and adult tissue is different from fetal tissue due to the fact that dividing cells dramatically slow down their division rate. In many cases, cells

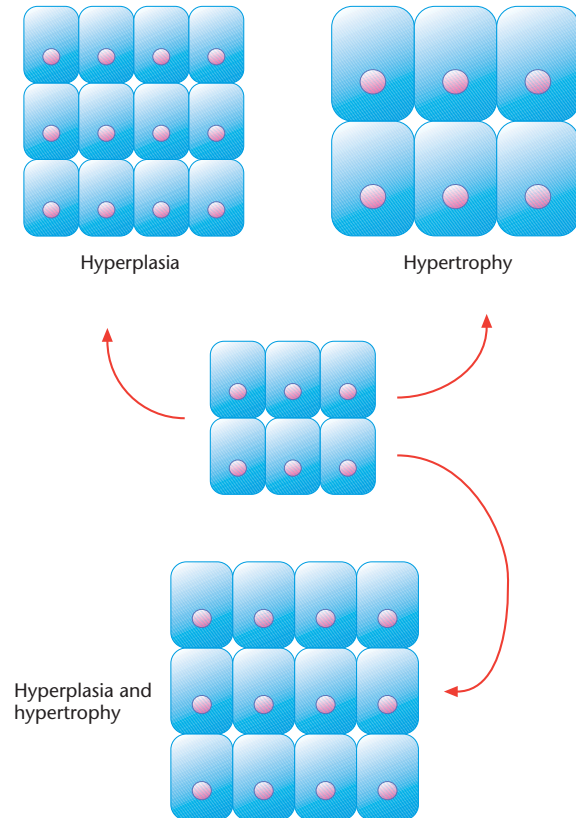


permanently exit the cell cycle and lose the ability to divide. Tissues in which cells maintain proliferative potential in adult life can easily replace injured or dead cells, and thus have a high regenerative potential. Cells of the haemopoietic system and lymphoid system, as well as skin and gut cells are some examples. Liver cells, on the other hand rarely divide but can be stimulated to if insult or damage occurs, thus maintaining some regenerative potential. Most neurons, cardiac muscle cells and skeletal muscle cells grow and divide during fetal development, but in post-natal life, the cells permanently lose the ability to divide and cannot be regenerated. To adapt to changing metabolic, functional or pathological demands, these tissues can only increase mass by increasing cell size. Both in normal growth and disease, this process is called hypertrophy.

## Hypertrophy versus Hyperplasia

Often during adult life, organs or tissues must adapt to different physiological conditions, or are challenged by a pathological condition. Such challenges may involve adjustments requiring an increase or decrease in the mass of an organ. **There are two general ways to increase organ mass: by increasing cell size (hypertrophy), or by increasing cell number (hyperplasia)** (Figure 5). Both methods of growth are common and occur normally, or as a result of disease. In many cases, growth is a combination of increasing cell size and increasing cell number. **Muscle hypertrophy is common in athletes who engage in demanding exercise.** Hyperplasia of breast and thyroid tissue occurs during puberty and pregnancy. Hyperplasia of epithelial cells is important for healing skin wounds. Similarly, hyperplasia of liver cells is important for repairing liver damage. In an experimental mouse model, cells of an injured liver, in which proliferation is blocked by a drug, will increase in size, suggesting that at least in this case, overall liver mass is critical and these cells adapt accordingly to maintain it. During puberty and pregnancy, uterine smooth muscle grows by a combination of hypertrophy and hyperplasia.

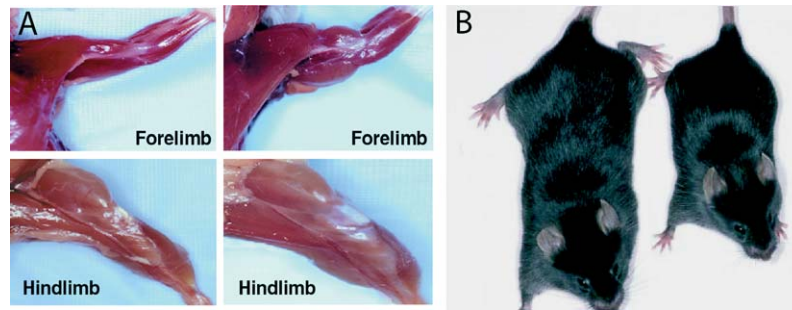
In tissues containing nondividing cells, individual cell growth becomes the critical regulatory mechanism of increasing organ size. **Hypertrophy of skeletal and heart muscle is common in athletes in response to increased exercise.** For example, runners, swimmers and wrestlers can have a left ventricle mass of over 300 g, while normal adult left ventricle mass is just over 200 g. As a result of increased load-bearing exercise, skeletal muscle fibres increase force generating potential by increasing the mass of individual myofibres. This occurs as a direct result of increased rates of protein synthesis. **A major molecule involved in muscle hypertrophy is the insulin-like growth factor IGF-1.** In the laboratory, researchers have shown using animal models that increased expression of IGF-1 is associated with skeletal muscle hypertrophy. In fact, forcing the expression of



**Figure 5** Hypertrophy versus Hyperplasia. Organs, during normal physiological growth, or when challenged by a pathological condition, can adjust in size in order to adapt to the changing demands. Increase in organ mass can occur by increasing the number of cells in the tissue, which is defined as hyperplasia. However, adult cells in some organs no longer maintain proliferative potential, and for such organs, increasing cell size is the only way to increase organ mass. This is defined as hypertrophy. Other organs use a combination of hyperplasia and hypertrophy to build mass. The increased size associated with hypertrophy directly results from increased protein synthesis.

IGF-1 specifically in mouse muscle tissue results in a dramatic increase in muscle mass compared to control mice that lack increased IGF-1 expression (Figure 6a).

Cardiac hypertrophy can develop as a result of myocardial infarction. Myocardial infarction causes the death of heart muscle cells by decreasing their supply of nutrients and oxygen. Cardiac myocytes, the major heart muscle cells, stop dividing after birth. Since myocardial cells cannot regenerate, the only way the surviving heart cells can compensate for the damaged cells and cope with the increased demand for work is to increase in size. This response is called left ventricular hypertrophy (LVH). LVH is often followed by pulmonary hypertension-induced hypertrophy of the opposite right ventricle, resulting in a severely enlarged heart. Cardiac hypertrophy can also result from hypertension, hormone imbalances or from genetic mutations.



**Figure 6** Effects of increased growth factor and hormone signalling on organ and organism growth in laboratory mice. (a) Skinned forelimb and hindlimb from a 6-month-old normal mouse (left) and a transgenic mouse (right) genetically engineered to express higher than normal levels of IGF-1 specifically in skeletal muscle. The transgenic mouse develops normally, but has dramatic hypertrophic muscles compared to the mouse expressing IGF-1 at normal levels. From Musaro A *et al.* (2001) (b) Both mice in this image are 2 months old, but the mouse on the left has been genetically altered to lack the normal regulatory systems to control growth. Compared to the normal mouse (right), this mouse has deregulated and overactive growth hormone and IGF-1 signalling, resulting in gigantism. From Metcalf D *et al.* (2000).

Contrary to increased organ size, atrophy is a decrease in organ size resulting from a decrease in cell size, cell number (by apoptosis) or both. Atrophy occurs as a normal physiological response during development and ageing, but can also occur pathologically. Atrophy can result from lack of use, as is the case for skeletal muscle during periods of prolonged limb immobilization. Hormone imbalances and nutrient deprivation can also induce atrophy in a variety of tissues. Skeletal muscle atrophy is common in patients with cancer, AIDS and sepsis. In the late stages of a terminal illness like cancer, severe nutrient deprivation results in cachexia, a condition of general weight loss and wasting. Decreased cell size associated with atrophy has been linked to increased protein destruction or proteolysis. Several molecules are reported to induce atrophy such as the cytokine interleukin-1 (IL-1), tumour necrosis factor (TNF) and transforming growth factor  $\beta$  (TGF- $\beta$ ). The fact that hypertrophy and atrophy are directly linked to controlling protein synthesis has focused attention on TOR as an important clinical target for treating such conditions. Modulating TOR activity might be a way to counteract the negative effects associated with hypertrophy or atrophy.

## How is Organ Size and Body Size Coordinated?

Remarkably, **the coordinated growth of our organs during development results in organ sizes generally proportional to body size in adults.** Proportions of some organs can vary during childhood. For instance, relative head to body size in children is larger than adults. Other examples include the reproductive organs, which grow minimally before puberty, but grow rapidly after puberty, and the lymphoid organs, which grow before puberty and then decrease their growth rate. There are also differences in growth rates be-

tween boys and girls. What coordinates the growth of organs with body size? Similarly, what controls the height to which individuals grow? Some critical factors that control body growth have been mentioned and include hormones and growth factors. Variations in systemic growth occur as a result of genetic factors, nutrition, environmental impact and disease, all of which can affect the production and effectiveness of growth factors and hormones.

Synthesis and secretion of hormones define the endocrine system of growth regulation. Hormones are synthesized and stored in glands, and released into the bloodstream where they travel to distant organs to regulate growth. The major hormone for regulating post-natal growth is growth hormone (GH). Growth factors additionally define the autocrine and paracrine systems of growth regulation. Growth factors can either feed back and regulate the cells that produced them (autocrine), regulate neighbouring cells (paracrine) or function as hormones travelling through the bloodstream to distant cells (endocrine). The key growth factors are those of the IGF family, including insulin, IGF-1 and IGF-2, with IGF-1 being the critical growth factor in post-natal growth. While growth factors bind to cell surface receptors, hormones pass through cell membranes binding to their receptors inside cells.

GH is synthesized in the pituitary gland. The concentration of circulating GH fluctuates because it is an unstable molecule with a half-life of roughly 20 min. Circulating GH stimulates the release of IGF-1 and -2 from the liver into the blood. Circulating IGF-1 and -2 are bound to proteins that stabilize them, resulting in a longer half-life and less fluctuation in levels. IGFs control bone and muscle growth, which have the greatest impact on body size. The importance of growth factor and hormone signalling in organism size control can be observed in laboratory models; for example, mice genetically altered to have excess growth signalling are gigantic compared to normal mice (**Figure 6b**).



Diseases affecting the pituitary gland can result in hypopituitarism and reduced GH production. This can result in dwarfism, in which the entire body is proportionally reduced in size. Similarly, reduced thyroid hormone, another hormone contributing to growth, specifically affects IGF-1 release from the liver. This condition also can result in dwarfism, however, it is disproportionate as the head remains normal in size and limbs are smaller because of reduced bone growth. Both conditions are treatable by supplementing hormones prior to puberty, during which time skeletal growth slows. Dwarfism also results from the rare disease Laron syndrome in which target tissues have reduced sensitivity to GH as a result of decreased GH receptors. Gigantism can occur from excessive GH production before puberty, often resulting from a pituitary tumour. Increased GH production after puberty (when skeletal growth slows) results in acromegaly, in which the hands, feet and head increase in size.

Is there a role for TOR in regulating body size? TOR is an intracellular effector of IGF-1, and since individual cells require TOR to grow, on one level the answer is yes. Eliminating TOR function in mice results in embryonic death, emphasizing the critical role of TOR in animal viability. But regulating protein synthesis and cell growth is only one critical function of TOR. Other evidence suggests TOR controls many cellular processes including cell proliferation, cell survival and some metabolic functions. Thus, TOR has a global role in mediating cell and organ growth as well as total body size.

Compared to adult growth, less is known about fetal growth. Maternal growth factors and hormones are not utilized in the developing fetus. In addition, fetal growth does not require a pituitary gland. Growth hormone is synthesized in the fetus, but it is not utilized in the same way. For the fetus, the critical mediator of growth is insulin. Insulin, in addition to its important role in regulating glucose metabolism, is required for initiating the production and release of fetal growth factors. Since insulin levels are regulated by blood glucose concentration, and blood can freely pass between mother and fetus, diabetic conditions can affect birth weight. Thus, a diabetic mother may have a heavy infant as a result of excess glucose in the blood.

## Genetics, Nutrition, Environment and Body Growth

Unless affected by a genetic or pathological condition, our bodies produce the necessary hormones and growth factors. Why then are we all different shapes and sizes? There are three major contributing factors to body growth regulation in humans: genetics, nutrition and environment. The most critical factor is genetic makeup. Genes passed from parents to their offspring determine the nature of the

complex biological processes controlling the synthesis and regulation of hormones and growth factors. Slight variations in growth genes as well as sex of an individual contribute to variations in body size.

Chromosomal abnormalities can reduce growth, as is observed in Down syndrome (trisomy 21) and Turner syndrome (XO females). The small stature of African pygmies may be linked to a chromosome abnormality leading to defective IGF-1 signalling. Beckwith–Weidemann syndrome (also known as exomphalos–macroglossia–gigantism syndrome) occurs sporadically and is linked to duplication of chromosome 11. Patients with this syndrome experience excessive growth of the tongue (which complicates breathing) and abdominal organs. Excess production of the insulin-producing pancreatic cells in Beckwith–Weidemann also causes hypoglycaemia as a result of excess circulating insulin.

Secondary to genetic control of growth is the role of nutrition and environment. Obesity is now a major health problem in some developed countries. While certain individuals may be genetically prone to weight gain, poor diet, overeating and lack of exercise has magnified the problem to epidemic proportions. In post-natal life, starvation, which is common in underdeveloped countries, can result in kwashiorkor. Initially, children with kwashiorkor become fatigued and lethargic, but prolonged starvation results in growth failure, decreased muscle mass, decreased immunity and the characteristic protuberant belly. Severe deficiency in protein and caloric intake can result in marasmus, a severe form of cachexia. The environment in which a fetus grows is also important as uterine size influences fetal size. Maternal factors such as smoking or drug and alcohol abuse also reduce birthweight, as does reduced oxygen availability to the fetus at high altitudes.

## Plant Cell Growth

Plant cells originate in specialized growth zones called meristems. The cells produced in meristems, located in roots and shoots, grow and proliferate and eventually differentiate to form organs. During organ development, plant cells cease dividing and enlarge up to 1000 times by a process called expansion. Cell growth in meristems and cell growth in organ development are achieved by fundamentally different mechanisms. Newborn cells in meristems are small ( $\sim 5\mu\text{m}$ ) and divide upon reaching a defined size. Growth in these proliferating cells is mechanistically similar to animal cell growth in that increased mass is achieved by increasing cell biomass (carbohydrates, proteins and lipids). The genetic analysis of cell growth in proliferating plant cells is in its infancy, but studies indicate many genes encoding TOR pathway growth regulators exist in plants.

During organ development, cells increase size by a different mechanism called expansion, a process by which water accumulates internally through osmosis. In addition

to having a plasma membrane, plant cells have a rigid cell wall surrounding the plasma membrane. The cell wall is primarily composed of cellulose. The cellulose content of plant tissues ranges from  $\sim 12\%$  in grassy plants, to nearly  $50\%$  in woody tissues, and up to  $\sim 90\%$  in cotton fibres, making it one of the most abundant macromolecules in plants. The cell wall is elastic, thus water influx causes plant cells to expand, but increased resistance from the cell wall eventually balances the osmotic pressure, a state defined as a cell's turgor pressure. This results in cell stiffening, which greatly increases a plant cell's load-bearing capabilities. A large internal organelle called the vacuole is important for regulating osmosis. The vacuole regulates the exchange of ions and organic molecules to increase osmotic pressure. The vacuole also stores waste since plants do not have specialized excretory systems. Growth by building biomass and growth through expansion are likely not completely independent processes as cells beginning expansion can still divide, while expansion can occur during the early stages of plant cell life. Moreover, endoreplication is also observed during expansion, thus plant cell growth is a dynamic and dramatic process.

Hormones such as auxins, gibberellins and cytokinins, direct the coordinated growth and proliferation of plant cells. Plant cell growth also responds to nutrient availability. Plants are not directly sensitive to amino acid starvation as they can generate their own amino acids, but require nitrogen and other minerals. Plants also reduce growth, and have the capacity to use alternative carbon sources and initiate autophagy in response to starvation. Thus, plant and animal cells grow by different mechanisms, but they share some basic principles with respect to regulating biomass accumulation and in coordinating organism growth during development and in response to starvation.

## Summary

What controls animal growth? In recent years it has become increasingly clear that cells have an intracellular mechanism to sense environmental growth conditions. If conditions permit growth, the signalling centre will trigger mass accumulation by initiating synthesis of new protein. TOR is a central component of this signalling centre and is directly responsible for activating the protein synthesis machinery. Thus, inhibitors of TOR may be promising agents for treating diseases of abnormal growth like cancer. Controlling growth is more complex in organs and organisms, where increases in cell number, cell size or both, as well as cell death, contribute to organ size. In some tissues, organ mass can only be increased by increasing cell size, called hypertrophy. Hypertrophy can result from a

pathological condition, as is the case for heart muscle after a myocardial infarction, while decreased muscle mass or atrophy is characteristically seen in patients with cancer and acquired immunodeficiency syndrome (AIDS). Plant cells, while employing fundamentally different mechanisms to grow, express TOR genes, suggesting some basic principles of growth may be conserved with animal cells. The widespread role of cell growth in a variety of human pathological conditions places TOR, a master regulator of growth, at the centre of focus for a variety of research programmes aimed at developing therapeutic agents to combat defects in growth.

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