Cell
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Review

The Regulation of Cell Size

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An adult animal consists of cells of vastly different size and activity, but the regulation of cell size remains poorly understood. Recent studies uncovering some of the signaling pathways important for size/growth control, together with the identification of diseases resulting from aberrations in these pathways, have renewed interest in this field. This Review will discuss our current understanding of how a cell sets its size, how it can adapt its size to a changing environment, and how these processes are relevant to human disease.

Introduction

The adult animal represents an endpoint of sorts, consisting of the surviving cells from the extensive growth, proliferation, and remodeling that takes place during development. Many of these cells continue to proliferate during adulthood, but one trait that distinguishes most adults from developmental stages is that the adult aims to remain more or less the same, hence the term homeostasis. In the adult, homeostatic mechanisms maintain cell number and size to preserve organ size and function. However, this outward appearance of stability belies the complex balance of positive and negative regulatory stimuli required to maintain tissues with the differing proliferative and metabolic activities that make up a complex organism.

In unicellular organisms such as yeast, cell growth and proliferation are mostly controlled by the extracellular nutritional environment, which allows a direct coupling of resources to cell generation. In multicellular organisms, however, growth, proliferation, and survival need to be differentially regulated in different tissues, so additional levels of control are required. This is achieved by providing a more or less constant supply of nutrients systemically (by the bloodstream or its equivalent), but in addition, there is a requirement by each cell for an instructive signal to grow, proliferate, and survive. Thus, a combination of multiple growth, mitogenic, and survival signals with cell-specific responses provides the diverse signaling required to produce and maintain a complex adult organism.

Although the signaling pathways and processes regulating cell proliferation and cell survival pathways have been intensively studied, until recently, the regulation of cell growth has received much less attention. Several new lines of investigation have reinvigorated this field of study. Studies showing that cells require extracellular instructive signals to grow, coupled with the identification of key signaling pathways, have provided tractable systems for studying how cell growth is regulated. Moreover, the identification of abnormalities in these pathways in diseases as diverse as cancer, cardiac hypertrophy and neurodevelopmental disorders have highlighted the critical importance of the tight regulation of these pathways and have identified potential new therapeutic strategies. In this Review, I will discuss our current understanding of how cells reach, retain, and adapt their size and how loss of these controls contributes to human pathologies.

Cell Growth versus Cell Proliferation

Cell growth (mass accumulation) and proliferation (cell division) are clearly separable processes. A cell can grow without dividing (for example, the growth of postmitotic neurons) and proliferate without growing (for example the cleavage divisions of a fertilized egg). Both processes require instructive signals, so a mammalian cell sitting in a culture dish surrounded by nutrients will not enter the cell cycle or add mass in the absence of a mitogen or growth factor, respectively (Conlon et al., 2001; Rathmell et al., 2000). These extracellular controls appear to be so stringent that, in the absence of a growth signal, a cell will "eat" itself rather than use the external supply of nutrients (Lum et al., 2005). However, despite both processes being regulated by extrinsic instructive signals, there are important differences between growth and proliferation that need to be considered to understand how they are regulated and coordinated.

Progression through the cell cycle tends to be an all-or-nothing, unidirectional process triggered by a threshold level of mitogenic signaling. Thus, although the rate at which cells progress through the cell cycle can vary, cells are either in the cycle as a result of mitogen stimulation or out of the cycle either because mitogen levels are too low or because the cell has permanently withdrawn from the cycle—for example, in terminally differentiated postmitotic cells. In contrast, most cells, whether in or out of the cycle—and many are permanently out of cycle for the entire adult lifespan—are constantly making and degrading macromolecules to maintain biological functions. The amount and type of biosynthetic activity can vary dramatically between cell types (compare rapidly dividing cells, nondividing secretory cells, metabolically active but postmitotic neurons, and quiescent oocytes). Yet, each of these cells is set to make and degrade macromolecules at a rate suitable for its needs to either maintain homeostasis or respond to a stimulus. A cell's size and growth rate is therefore determined by the balance between the rates of accumulation of macromolecules (by synthesis and uptake) and their loss (by degradation and secretion), which can vary in a graded fashion in response to changing levels of growth factor signaling.

The notion that seemingly quiescent cells may actually be highly biosynthetically active was dramatically shown in a recent study of mammalian fibroblasts in which biosynthetic activity

was compared between fibroblasts removed from the cell cycle by contact inhibition and rapidly proliferating cells (Lemons et al., 2010). Remarkably, the contact-inhibited cells continued to synthesize proteins at the same rate as the proliferating cells, even though the contact-inhibited cells were apparently producing much less net mass over a similar period of time. The explanation was that contact-inhibited cells had changed their metabolism, increasing the amount of protein degradation and secreting large amounts of extracellular matrix proteins. Although these findings probably reflect the differing physiological roles of dividing and nondividing fibroblasts, they are also a potent reminder that intuitive assumptions about the biosynthetic rates of different cells may be highly inaccurate and that nongrowing, nonproliferating cells can be as biogenic as growing and proliferating cells.

Establishing and Maintaining Cell Size

The size of an adult organism is determined by both intrinsic developmental programs and by extracellular signals, which integrate to control cell number and cell size. Differences in animal size are mostly genetically determined and primarily reflect differences in cell number rather than differences in cell size (Conlon and Raff, 1999). However, despite the more or less fixed target size of most organisms, external signals can still impinge on this genetic program. One clear example of this is the effects that nutrient levels can have during development. When in excess, nutrient levels do not appreciably affect maximal organismal size, but when limited, they can have a dramatic effect. For example, it has been shown that extreme nutrient deprivation during development can decrease a fly's size to 15% of normal (Edgar, 2006).

Thus, at least in well-nourished adult animals, tissue size tends to have been set by the balance between the proliferation and survival rates of stem and progenitor cells that established the tissue and the timing of when these cells left the cell cycle or when they reached a homeostatic state. However, this is not always the case. Pioneering experiments in *Drosophila* showed that inhibiting cell division within half a developing wing disc had little effect on the final size of the wing as the cell-cycle-arrested cells grew larger (Su and O'Farrell, 1998). Similarly, whereas pancreatic size is controlled by progenitor cell number, liver size is not, indicating that the final size of a tissue can be determined by its total cell mass rather than cell number (Stanger et al., 2007).

The size of the cells within a tissue will be the "readout" of their growth and proliferation rates, both during development and in the adult, which are controlled by intrinsic programs and the levels of extracellular mitogens and growth factors, as well as other factors that can impinge on these pathways such as nutrient levels, mechanical signals—which can act both positively (such as stretching during periods of growth) and negatively (for example, crowding within a tissue)—and stress (Conlon and Raff, 1999; Tumaneng et al., 2012a). The lack of a fixed cell-sizing mechanism and the separable and independent regulation of cell growth and proliferative pathways have been demonstrated in multiple cell types and are shown most clearly by the repeated finding that increasing the growth rate of cells usually has little effect on cell number but can dramatically increase cell size and hence tissue size (Edgar, 2006; Jorgensen and Tyers, 2004).

On reaching adulthood, tissues and cells mostly maintain their size. This homeostatic maintenance of form is seen in rapidly renewing epithelial tissues, postmitotic cells such as neurons and muscle, and regenerative cell types such as liver, endothelial, and Schwann cells, which can maintain their size for years but retain the capacity to renter the cell cycle, proliferate, and form new tissue of the appropriate size. The robustness yet flexibility of the homeostatic state is likely to require robust but responsive regulatory networks, and defects of these controls will likely contribute to disease. Remarkably, these regulatory networks remain poorly understood even in possibly the simplest situation to consider, which is the maintenance of the size of a nondividing cell in the adult. As discussed above, a nondividing adult cell that maintains a constant size is not in the absence of a growth signal (it would atrophy) or biosynthetically inactive (it would fail to function) but rather in a balanced state resulting from a defined level of growth pathway signaling. In this homeostatic state, the rates of synthesis and degradation of macromolecules are balanced to result in no net change in the mass or volume of the cell. Moreover, the water content of the cell must be controlled, requiring stringent controls of osmotic pressure (Koivusalo et al., 2009). This is all the more remarkable when considering the highly dynamic nature of most cells and the rapid turnover of many cell components and organelles, yet this maintenance of cell size can last a lifetime. An indication of the controls involved and the importance of growth factors in their regulation were demonstrated by studies in sensory neurons, which concluded that synthesis and degradative pathways are coupled in order to maintain cell size. In these experiments, the neurons were treated with the neurotrophin NGF (a growth factor for these cells) in the presence of increasing levels of an inhibitor of protein synthesis. Incredibly, cell size was maintained because of a proportional decrease in the degradative rates of long-lived proteins. In contrast, in the absence of NGF, the cells shrank (Franklin and Johnson, 1998). This coupling of protein synthesis and degradative pathways could be a general mechanism to provide robust homeostasis in the face of likely fluctuations in growth factors, nutrient levels, or cellular damage.

Growth Pathways—Controlling the Growth Rate

Where significant progress has been made in recent years is the identification of many of the key regulatory pathways that control cell growth. The best-characterized example of which is the IGF/PI3K/AKT/mTORC1 pathway (Figure 1). This evolutionary conserved pathway has been shown to be a major regulator of cell growth and thus a key determinant of cell size; moreover, artificial activation of this pathway can promote additional growth in most cell types tested (Edgar, 2006; Laplante and Sabatini, 2012; Tumaneng et al., 2012a). IGF is a classic example of a limiting growth factor that acts both systemically and at local tissue levels. Overexpression during development results in larger animals, mainly due to increases in cell size, and overexpression in the adult can result in cell hypertrophy. Binding of IGF to its receptor activates multiple signaling pathways, but key to regulating cell growth is the activation of the PI3K/AKT/mTORC1 axis with mTORC1, a central mediator of the signal

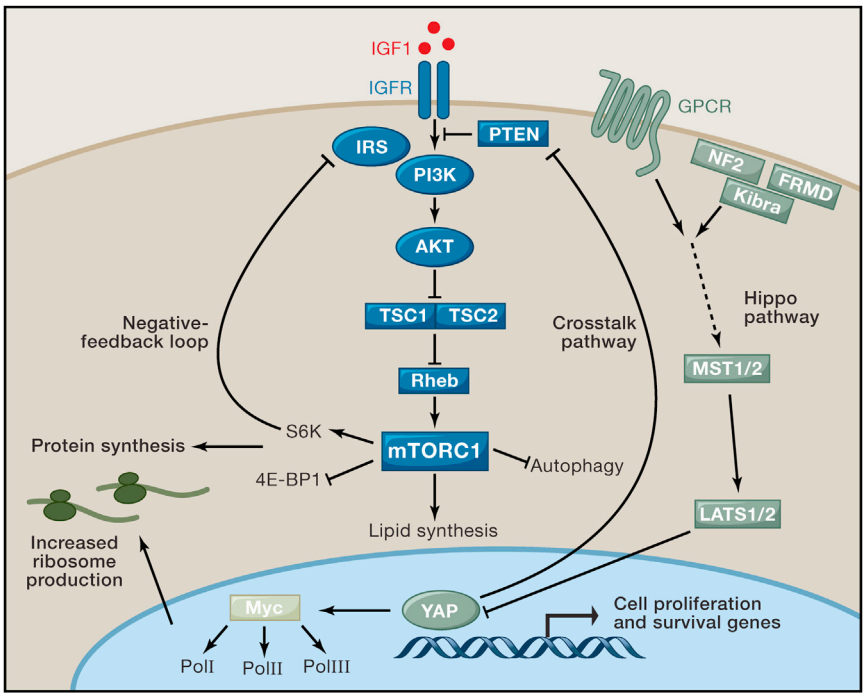

Figure 1. Growth Pathways

A highly simplified cartoon outlining the major known signaling pathways that regulate cell growth. For more mechanistic details, please see the following excellent reviews: Laplante and Sabatini (2012), van Riggelen et al. (2010), and Yu and Guan (2013).

from the growth factor to biogenic pathways. In addition, mTORC1 integrates inputs from at least four other major cues that can affect cell growth—stress, energy status, oxygen, and amino acid levels—and thus acts as a signaling node at which energetic and stress signals can modulate growth factor signaling (Laplante and Sabatini, 2012). For example, in the absence of amino acids, IGF is unable to activate mTORC1 signaling. Although full amino acid deprivation is unlikely to occur in vivo, as animals aim to maintain relatively constant levels of metabolites, nutritional levels are likely to contribute subtly and have cell-specific roles in signaling through this pathway. Increased signaling through the mTORC1 pathway promotes multiple biogenic processes, including nutrient uptake and protein and lipid biosynthesis, and modulates cellular metabolism to promote biogenesis; it also inhibits catabolic pathways such as autophagy (Locasale and Cantley, 2011). Importantly, mTORC1 activates a potent negative-feedback loop that, via IRS, acts to negatively regulate signaling by the IGF receptor, providing an example of how a biogenic pathway can be buffered to contribute to cell size homeostasis.

Recently, however, it has become clear that mTOR is not required for the growth of some cell types, and these cell types seem to be those that are spared in response to nutrient deprivation (Cheng et al., 2011). Neuroblasts in developing *Drosophila* continue to grow in starvation conditions, whereas other "lower priority" tissues are growth restricted to various extents. The growth of these cells is independent of dTOR, yet requires both PI3K/AKT signaling and activation of the dTOR targets S6K and 4E-BP, indicating that these growth pathways have been "rewired" to make them less sensitive to nutrient levels. Moreover, these cells are also insensitive to decreasing levels of circulating insulin-like peptides because the receptor tyrosine kinase ALK is kept active by a ligand expressed by the surrounding niche (Cheng et al., 2011).

Another major regulator of biogenic pathways is the transcription factor Myc. Myc increases cell growth and cell size in multiple tissues and organisms, and this is associated with increased ribosomal RNA (rRNA) levels, nucleolar size, increased protein biogenesis, and the metabolic reprogramming required for cell growth (Grewal et al., 2005; Saucedo and Edgar, 2002; van Riggelen et al., 2010; Wang et al., 2011). Importantly, Myc- and PI3K-dependent cell growth appears to be driven in part by distinct mechanisms. In *Drosophila* fat cells, overexpression of PI3K and Myc causes an equivalent increase in cell volume. However, the Myc cells appear more protein and ribosome dense with an increase in nucleolar volume compared to the more lipid-rich PI3K-expressing cells (Saucedo and Edgar, 2002). This indicates that these two growth-promoting pathways differentially activate biogenic pathways, perhaps reflecting the requirement of cells to "grow" in different ways depending on their function.

The Hippo pathway is also important in the control of tissue/organ size, mainly by regulating proliferation and apoptosis and thereby cell number (Tumaneng et al., 2012a). A major downstream effector of this pathway is the transcriptional coactivator YAP1, which activates genes that promote proliferation and protect against apoptosis. Activation of YAP1 in postnatal liver leads to a massive expansion of the tissue due to an increase in cell number; if YAP1 is then switched off, the liver returns to normal size as the excess cells die by apoptosis. Thus, the Hippo pathway not only controls the production of cells but sustains a level of mass perhaps by coordinately controlling both the proliferative and survival pathways (Dong et al., 2007). Producing and sustaining this mass, however, should also require increased signaling through a biogenic pathway. Perhaps not surprisingly, recent work in both *Drosophila* and mammals has shown crosstalk between the Hippo and mTOR signaling pathways (with YAP1 activating mTOR by decreasing PTEN levels) and Myc, hence providing mechanisms whereby organ size can be determined and maintained by the coordinated regulation of proliferative, survival, and growth pathways (Csibi and Blenis, 2012; Neto-Silva et al., 2010; Tumaneng et al., 2012b).

Although it is clear that these growth pathways are important in all tissues tested, their roles in the maintenance of cell size are less clear. For example, some organs, such as liver and

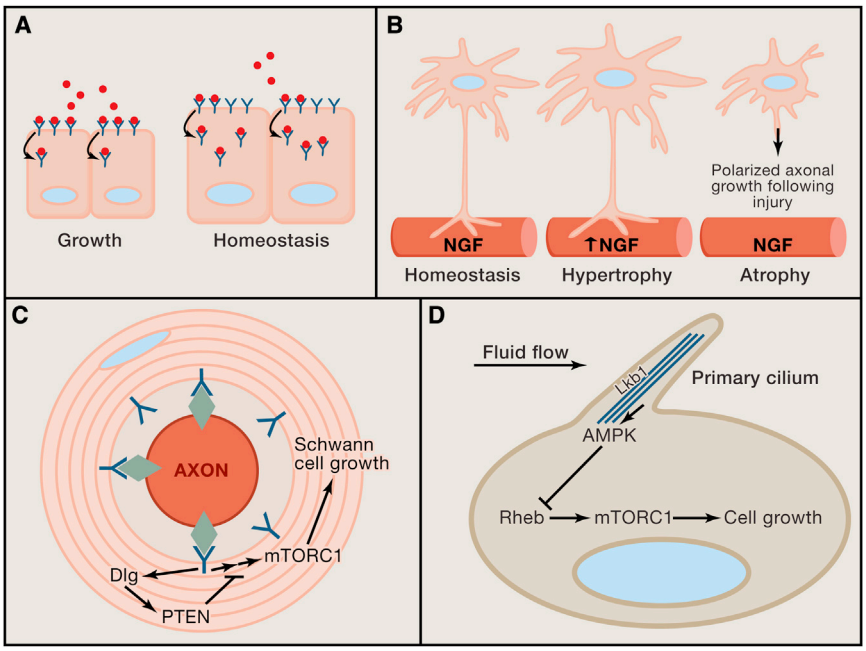

Figure 2. Extracellular Signals that Regulate Cell Size

(A) A set level of growth factors can allow cells to grow to a specific size. One mechanism may be that, as a cell grows, it ingests and degrades more growth factor until an equilibrium is achieved between the level of factor and the size of the cell.

(B) The amount of NGF produced by the target tissue controls the size of the neurons that innervate the target.

(C) The amount of ligand (green diamond) is proportional to the size of the axon and controls the growth of the surrounding Schwann cell.

(D) Flow is detected by the primary cilium on kidney epithelial cells and negatively regulates cell size.

muscle, atrophy following inhibition of mTOR. In contrast, inhibiting mTOR activity in the adult prostate or in postnatal granule neurons of the cerebellum has little detectable effect on the size of these cells over substantial periods of time (Kwon et al., 2003; Nardella et al., 2009). They are not restrained at a particular size, however, as each can increase in size in response to a growth signal, and this growth is mTOR dependent, showing a differential requirement for mTOR in the growth versus maintenance of cell size. How cell size is maintained in these cells and how and if the metabolism of the cell is adapted to cope with the loss of this fundamental signaling pathway remain to be determined.

Does a Cell "Know" Its Size?

There is little evidence that a cell directly senses or uses some type of ruler to measure its size. Instead, cell size reflects a homeostatic level of signaling that produces a balance of anabolic and catabolic processes that maintain the size of both nonproliferating and proliferating cells. Yet, although cell size is mostly stable and predictable, it can be changed, indicating that a cell is constantly responsive to signals that establish and maintain its size. But what are the signals that set and maintain cell size? Although mostly poorly characterized, there are certain cell-specific examples that indicate how cell size can be established and that illustrate the importance of both systemic signals and local signals, as would be expected in order to coordinate the complex tissue architecture found in multicellular organisms.

It is clear that both systemic and locally acting extracellular growth factors such as IGF1 can control cell size. These factors are often limiting and/or produced at higher levels during periods of growth. Therefore, simple models can be constructed in which a certain level of growth factor will set a certain cell size. For example, as a cell increases in size, the capacity to degrade the growth factor may also increase until the level of growth factor is lowered to a level that produces a stable cell size (Figure 2A).

For some neurons, cell size can be determined by the level of growth factor produced by the target tissue (Figure 2B). Postmitotic neurons often extend very long axons to innervate their target tissue, a growth process stimulated by local neurotrophins and other signaling molecules that control the guidance of the axon (Bellon et al., 2010; Chilton, 2006). Upon target innervation, signals from the target tissue inhibit further axonal extension (Moon and Birren, 2008), but then the level of growth factors expressed by the target tissue determines and maintains the size of the neuron (Fawcett and Keynes, 1990; Purves et al., 1988). This has been particularly well established for sympathetic neurons for which the level of NGF in the target tissue sets the size of the cell. During development, increasing or decreasing NGF levels can increase or shrink cell size, a mechanism that allows the neurons to increase in size in a coordinate fashion with the increasing size of a target tissue as the animal matures (Figure 2B). Moreover, this quantitative regulation persists in the adult, as manipulation of the target size or NGF levels in the adult can still cause corresponding changes in neuronal size, demonstrating that NGF both determines and maintains the size of the cell. This size maintenance, although robust, is also adaptable because, following injury, the neurons can regenerate. Cutting the axons results in a separation from the target tissue, and the resulting loss of the homeostatic NGF signal causes dendritic retraction and cell body shrinkage. However, polarized growth is reinitiated in the axon, presumably in response to locally produced growth signals at the injury site until reinnervation re-establishes the NGF signal and the neuron is restored to its original size.

A further example of how heterotypic cell interactions control cell size is also provided by the peripheral nervous system (PNS). Schwann cells interact with all neurons in the PNS, but they only myelinate those with a diameter greater than 1 μm. This requires the Schwann cell to "measure" the axonal diameter and make a distinct differentiation decision based on this measurement. Moreover, the ratio between axon diameter and myelin

sheath thickness (the g ratio) is fixed at an optimal value that achieves the most efficient nerve conduction. This means the larger the axon, the larger the Schwann cell and requires coordinated growth as the axons continue to grow as the animal matures (Roberts and Lloyd, 2012). The level of a membrane-bound ligand neuregulin1 (NRG1) type III presented by the axon controls both whether an axon is myelinated (a threshold response) and also the thickness of the myelin sheath (a dose-dependent response). The larger the axon, the more NRG1 type III is expressed, whereas the responsible receptor (the ErbB2/3 complex) appears to be in excess, so that increasing numbers of receptors are likely to be activated in response to a larger axon. The receptor activates both a biogenic pathway (the PI3K/AKT/mTOR pathway) and a negative-feedback loop (increasing the levels of the scaffold Dlg1 that act to stabilize PTEN), which are both important for stabilizing the size of the Schwann cell at a given level of the NRG1 signal, and provides a model for how a different level of receptor signaling can result in a new steady-state size rather than in perpetual growth (Figure 2C).

A rather different type of signal that regulates cell size has been identified in the kidney (Boehlke et al., 2010). In polycystic kidneys, the cells lining the tubules are larger than normal. These genetic diseases are associated with defective cilia, and a number of other mutations that disrupt primary cilia also result in an increase in the size of these cells. Fascinatingly, the role of cilia in this case is not to sense the level of an extracellular factor but to detect urine flow and transduce this signal to the growth pathways. This involves a mechanism by which the kinase Lkb1, which is localized within the cilium, is activated by flow to increase the activity of AMPK, a negative regulator of the mTOR pathway (Figure 2D). Thus, increased flow should maintain a smaller cell size and contribute to efficient flow through the tubules. This example of how a mechanical signal can contribute to cell size regulation demonstrates the myriad of signals that need to be considered in understanding how cell size may be controlled.

Changing the Size of Nondividing Cells

Theoretically, a cell can increase in size by several mechanisms. The biosynthetic rates can increase, the degradative rates can decrease, or both rates can change but the ratio between the synthetic and degradative rates increases. At first thought, it may seem that the most energetically expedient way for a cell to grow would be to increase the synthesis rate while decreasing the degradative rate. However, in the rare cases in which this has been measured, it does not appear to be the case in that, following a growth stimulus, both synthesis and degradative rates of proteins often increase but the synthetic rate increases more, resulting in a net increase in protein mass (Conlon and Raff, 2003; Tipton and Wolfe, 1998). One obvious reason why degradative rates will increase with any increase in synthesis is because ~30% of all polypeptides are rapidly degraded in the proteosome following synthesis, with this "molecular triaging" representing ~75% of all proteosome substrates (Schubert et al., 2000).

Most nondividing cells don't significantly change their size upon reaching adulthood. However, one cell type that can change dramatically in size in the adult is the muscle cell (myocytes). Following exercise, particularly mechanical-load-incurring exercise, adult muscle will increase in size as a result of individual cells growing in the absence of proliferation of either the myocytes themselves or the muscle stem cell population (satellite cells) (Braun and Gautel, 2011). Conversely, in adverse conditions such as starvation, disuse, or wasting pathological states, individual myocytes atrophy. Although muscle, like all tissues, is highly specialized, studies on the changes in size of this tissue give clues to how cell size can both be maintained and can change during adulthood.

Two major pathways have been shown to be important for regulating the size of adult skeletal muscle. On the anabolic side is the IGF/PI3K/AKT/mTORC1 pathway, which controls multiple biogenic pathways, including protein translation. On the catabolic side is the myostatin/SMAD2/3 pathway (Otto and Patel, 2010). Myostatin is a member of the TGFβ family, is secreted by muscle, and is thought to act mostly locally to negatively regulate muscle mass. Mutations in the myostatin gene have been shown to lead to a massive increase in muscle size in multiple species; indeed, cows selected for larger muscles contain mutations in this gene. Much of the effects of myostatin have been shown to take place during development by negatively regulating the proliferation, differentiation, and growth of muscle progenitor cells; however, myostatin is still produced in the adult and inhibition in adult muscle results in hypertrophy, showing that the pathway continues to inhibit adult muscle growth (Otto and Patel, 2010; Sartori et al., 2009). Similarly, inhibition of the AKT/mTORC1 pathway in the adult results in muscle atrophy, whereas activation of the pathway results in hypertrophy (Schiaffino et al., 2013). There is negative feedback regulation in both pathways and crosstalk between them, with much of the ability of myostatin/SMAD2/3 to inhibit cell growth being dependent on modulation of AKT/mTOR signaling (Figure 3) (Sartori et al., 2009; Trendelenburg et al., 2009; Winbanks et al., 2012). The size of adult muscle cells will thus be the result of a balance between these two pathways, resulting in a synthesis and degradative balance such that there is no net growth.

Following exercise, there is an increase in mTORC1 signaling that appears to be mostly independent of PI3K/AKT and involves force/stretch signaling mediated by mechanosensors embedded in the sarcomere (Bodine et al., 2001b; Miyazaki and Esser, 2009). In response to this signal, it appears that both protein synthesis and degradative rates increase, but synthetic rates increase disproportionally, resulting in muscle cell hypertrophy (Biolo et al., 1995; Tipton and Wolfe, 1998). A new size is reached, with further increases in size produced following further bouts of exercise. Interestingly, however, this increase in mass is not maintained unless the exercise is maintained (note the gym maxim "use it or lose it"), indicating the importance of continuous signaling through the mTOR pathway to maintain muscle size.

A further interesting observation is that, following a constitutive increase in signaling through the AKT/mTOR pathway, muscle cells increase in size (as would be expected following an increase in signaling through biogenic pathways) but do not continue to grow; rather, they stop at a larger size (Lai et al., 2004). This requires constant elevated signaling through this pathway but must also involve negative regulators (a parallel

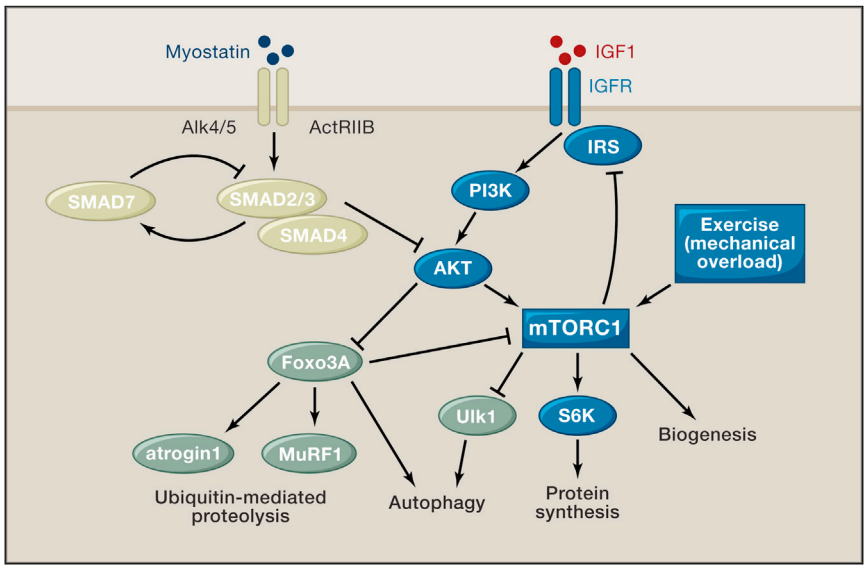

Figure 3. Regulation of Skeletal Muscle Size

Simplified cartoon of the main signaling pathways that maintain and can change the size of skeletal muscle. Biogenic pathways are shown in blue, negative regulators are shown in cream, and the pathways activated during muscle atrophy are shown in green. Negative-feedback loops of both pathways are indicated.

increase in degradative pathways, for example) that will result in the maintenance of a larger cell size rather than continuous growth.

Following starvation and in other pathological states, muscles can rapidly lose mass as a result of an increase in specific degradation pathways, which appear to have a minor role in maintaining normal muscle size. In this situation, proteins are targeted for degradation by both the ubiquitin-proteosome system and the lysosomal autophagic pathway. The regulation of the genes involved in triggering these processes—the so-called atrophy-related genes—are under the control of the AKT and mTOR signaling pathways, and these genes are switched on when the level of activity of the pathways drops below the basal homeostatic signaling level (Bodine et al., 2001b; Castets et al., 2013; Stitt et al., 2004). In particular, two of these genes, the ubiquitin ligases astrogin-1 and MuRF1, are regulated by FOXO3A, a transcription factor negatively regulated by AKT (Zhao et al., 2007) (Figure 3). Knockout of these genes produces mice with normal muscle, showing that the genes are not important in regulating normal muscle cell size, but the muscle is resistant to multiple signals capable of inducing muscle atrophy (Bodine et al., 2001a). The atrophy response most likely reflects a stress pathway by which muscle breakdown can transiently provide amino acids to the rest of the body; nevertheless, following refeeding, muscle cells return to their normal size.

Maintaining and Changing the Size of Proliferating Cells

The majority of studies on cell size control have been performed on rapidly proliferating populations of cells. These include exponentially proliferating populations of yeast cells or mammalian cancer cells, as well as developmental systems in which there is rapid cell proliferation such as the imaginal discs of the fly. These cell systems have evolved to achieve rapid cell growth and proliferation, and they make tractable systems for addressing many important questions about cell size control. It is useful to bear in mind, however, that in the adult mammal, this type of sustained, exponential proliferation is very rare. Therefore, these findings may have limited relevance.

Observations of proliferating cells revealed that cell growth and cell division appear to be tightly coordinated in that cells usually double their mass before each division and, as a result, maintain a constant mean size over time. Although this apparent coordination might just reflect the "read-out" of specific but separable growth rates and cell-cycle times, theoretical considerations led to the hypothesis of a direct regulatory link between cell growth and the cell cycle, which came to be known as the "cell size checkpoint." The theoretical requirement for a proliferating cell size checkpoint rested upon whether the addition of mass in an individual cell occurs in a linear or nonlinear manner (Conlon and Raff, 2003; Mitchison, 2003). Initial experiments in yeast determined that growth during the cycle is in fact exponential in that, as a cell gets bigger, it adds mass at an exponentially greater rate, a finding that is easy to reconcile with an increase in ribosomes and other biogenic machinery as a cell increases in size (Elliott and McLaughlin, 1978). However, if growth in an individual cell is exponential, it means that, in a population of cells with a spread of cell sizes, the bigger cells within the population would add more mass over time than the smaller cells so that the size range of the population would increase over time. That this does not happen led to the suggestion of a process that can act to limit this divergence in size, and the search for this elusive "cell size checkpoint" has continued ever since. In contrast, if cells add mass in a linear fashion, cells would not require a sensing of size or a checkpoint mechanism to limit a divergence in cell size, but an equally mysterious mechanism would be needed to "measure" the addition of the same amount of mass in a manner that is independent of the size of the cell.

Whether cells grow in a linear, exponential, or other manner and how cell size may be linked to the cell cycle remains controversial. Recent studies in yeast—in particular, the budding yeast *S. cerevisiae*—have re-explored these issues using single-cell analysis of mother and daughter cells as they transit through the cell cycle. Mother cells are much larger than daughter cells, and as daughter cells have been shown to progress through G1 more slowly than mother cells, this led to the notion that they needed to reach a critical size before passing Start, the decision point for entry into S phase (Turner et al., 2012). Several findings are clear from these and some earlier studies: (1) there is not a critical cell size or "Sizer" that is the trigger for entry through

Start, as the daughter cells pass through at variable sizes (Lord and Wheals, 1981; Wheals, 1982); (2) there is no correlation between the size of the mother cell and the time to passage through Start, implying that once over a certain size, a timer mechanism is employed (Di Talia et al., 2007; Lord and Wheals, 1983); and (3) there is a size-independent mechanism that explains why daughter cells pass Start more slowly than mother cells—as daughters still enter Start more slowly than similarly sized mother cells—which appears to be due to asymmetric distribution of transcription factors that negatively regulate the cell cycle (Di Talia et al., 2007, 2009; Turner et al., 2012). However, there does appear to be a degree of size correlation between the daughter cells in the early period of G1 that suggests that the size of smaller cells is influencing the duration of passage through this period of the cell cycle. The mechanism is not clear but has been linked to the levels of the cyclin CLN3, which controls passage through this early part of the cell cycle. However, although there does appear to be some correlation with size, the major cause of the variability in the time to passage this part of the cell cycle appears to be size independent and instead reflects transcriptional noise that causes cell-to-cell variability in the levels of the lowly expressed CLN3 transcript (Di Talia et al., 2007).

Interestingly, two recent studies in *S. pombe*, a fission yeast, identified a direct mechanism that the cell uses to measure cell size that does directly impinge on the cell cycle (Martin and Berthelot-Grosjean, 2009; Moseley et al., 2009). *S. pombe* grow in a lengthwise manner, with mass added to the end or ends to create an increasingly longer cell as it progresses through the cycle. The localization of a dose-dependent inhibitor (Pom1) of the G2/M transition at the ends of the cell creates a spatial gradient whereby the level of the inhibitor decreases in the nucleus as the cell elongates, thus directly linking the size (length) of the cell to entry into mitosis. Whether this mechanism is sufficient to control the size of proliferating cells and whether its use is limited to small, elongating, rather rigid cells remain to be clarified.

In mammalian cells, some studies have found that cell growth is linear, with both synthesis and degradation rates apparently increasing with cell size, providing a possible mechanism for adding a fixed amount of mass per unit of time, independent of cell size (Brooks and Shields, 1985; Conlon and Raff, 2003; Hutson and Mortimore, 1982). Other studies have found that growth is not linear and argue for the existence of a size checkpoint, although the mechanisms have remained unexplained (Dolznig et al., 2004; Zetterberg and Killander, 1965). Recently, a number of increasingly sophisticated methods to measure the mass and volume of individual cells as they progress through the cell cycle have been used to re-explore this issue (Godin et al., 2010; Kafri et al., 2013; Mir et al., 2011; Park et al., 2010; Son et al., 2012; Tzur et al., 2009). These studies have yet to provide a definitive answer, but they hint at greater complexities than were initially envisaged. Although not able to demonstrate either simple linear or exponential growth through the cell cycle, cells seem to increase their growth rate as they progress through the cell cycle; this increase is most obvious once cells have entered G2, perhaps because of the doubling of DNA in S phase. Interestingly, there appears to be a slowing of the growth rate as cells enter S phase, with a greater slowing in larger cells with a faster growth rate (Goranov et al., 2009; Kafri et al., 2013). This differential slowing could act as a mechanism to limit cell size variability, thus limiting the divergence in cell size without the need for a classical size sensing mechanism or checkpoint. A further possibility is that the slowdown in growth rate on entering S phase could reflect energy depletion associated with the sudden onset of the energetically expensive process of DNA replication, which might affect faster growing cells preferentially and may have less of an effect on cells proliferating in a more pedestrian manner or in more amenable nutrient conditions.

Another important consideration is that, if there is a cell size checkpoint in yeast and/or mammals cells, it must be adaptable, i.e., there cannot be a fixed intrinsic determinant that permits or triggers passage through the cell cycle at a specific cell size. This is because it is possible to vary the size of cells. For example, yeast are different sizes in distinct nutrient conditions, and it is easy to dramatically change the size of proliferating mammalian cells by simply varying mitogen and growth factor levels (Conlon et al., 2001; Echave et al., 2007). This variation in size also rules out a direct readout of growth rate rather than cell size to regulate entry into the cell cycle, as cells growing at different rates (such as yeast in rich versus poor medium) should maintain their size but just have a shorter cell cycle, which is not the case. In multicellular organisms in vivo, this simple mechanism to alter cell size by responding to changes in levels of extracellular growth factors and mitogens seems likely to be the predominant mechanism for controlling the size of proliferating cells in the animal. This mechanism is consistent with in vitro studies and multiple genetic studies in flies and mice. Importantly, it would provide the flexibility to create the complex tissues, filled with cells of a variety and changing set of sizes, that are found within multicellular animals.

Minimal and Maximal Cell Sizes

Related to cell size checkpoints are possible minimal and maximal limits to cell size. There appear to be limits at both extremes, but whether these constraints can be considered checkpoints and what their physiological relevance may be remain unclear. There is obviously a minimal limit to the size of a cell that is viable or that can enter the cell cycle, as a cell requires a minimum set of components to exist. Interestingly, in the Schwann cell, this lower limit seems surprisingly small in that, when cultured in the mitogen neuregulin but in the absence of growth factors, the cells continue to proliferate until they are so small that they no longer adhere to the dish (P. Echave and A.C.L., unpublished data; Conlon et al., 2001). However, there are examples in which very small cells appear to have to grow to a minimal size before they can enter the cell cycle. B lymphocytes, genetically engineered to survive in the absence of survival factors, shrink to an extremely small size following the removal of their growth factor, IL3, (which is also a mitogen); upon readdition of IL3, there is a clear lag, as the cells grow to a minimal size before entering the cell cycle (Lum et al., 2005). This in vitro finding mirrors the situation seen in various developmental situations when small cells produced by division in the absence of growth grow to a minimal size before re-entering the cell cycle. A clear example of this is seen during neuroblast development in *Drosophila*, when, following rapid early divisions

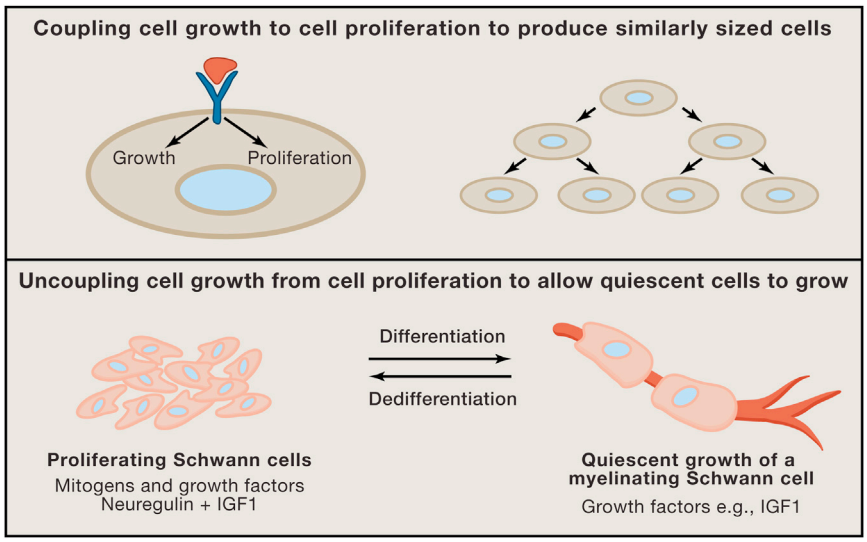

Figure 4. Independent Regulation of Cell Growth and Cell Proliferation

leading to the production of very small cells, there is a lag during which the cells grow to a larger size before entry into the next rounds of divisions (O'Farrell, 2004).

Cell can also reach very large sizes. In particular, some ova can be 1 mm in diameter, whereas nerve cells can be 1.5 m in length in humans and up to 12 m long in the giant squid. However, these very large cells tend to use highly specialized mechanisms to allow them to grow so large. For example, neurons have specialized intracellular transport systems and use support cells to gain nourishment away from the cell body. In vivo, it appears that most cells are not at their maximal size as they can get bigger following an increase in growth stimulation. In vitro, in the presence of saturating levels of growth factors, however, cell size plateaus at a maximal size. Whether this maximal size merely reflects a new steady-state size in response to the saturating levels of growth factors or whether there are real spatial or structural limitations remains unclear. It is easy to envisage numerous possible limitations: a decrease in surface area/volume ratio as cell size increases could limit nutrient uptake or a limit to transcription or translation could stop further growth. Indeed, the saturation of any biogenic pathway could limit cell size.

A limiting factor that is likely to be important is the ploidy of the cell. The evidence for cell ploidy affecting cell size and limiting cell growth is manifold. First, it has been known for decades that increasing the ploidy of a cell usually, but not always, increases the size of the cell proportionally (Lee et al., 2009). For example, tetraploid cells are twice the size of diploid cells. This does not necessarily mean that the size of a cell is limited by the genome. It could just reflect that doubling the output of everything from receptors to ribosomes to genes results in a doubling of cell size. It does, however, show how cell size can be influenced by output from the genome. More compelling is the finding that, in many organisms, large cells or very active cells tend to have undergone endoreduplication, suggesting that an increase in ploidy is required either to support a larger cell or to maintain a highly biosynthetically active cell (Lee et al., 2009; Umen, 2005). In mammals, such cells include liver cells, heart muscle cells, and trophoblasts, and these endoreduplication cycles appear to be important for function. In both *Drosophila* and *C. elegans*, many cells undergo endoreduplication to produce extremely large cells: for example, a whole muscle in *Drosophila* can consist of a single endoreduplicated cell. Genetic studies show that endoreduplication is required for the *Drosophila* cells to grow so large, but whether endoreduplication alone is sufficient to drive growth is not clear and may vary between tissues. In fly muscle, it appears not to be sufficient, because although myc induction is sufficient to trigger polyploidy, it is not sufficient to drive substantial cell growth. In contrast, in the fat body, there is a correlation between myc induction of polyploidy and cell growth, which is more consistent with an increase in polyploidy driving cell growth but could also indicate that myc can efficiently drive both processes in this tissue (Demontis and Perrimon, 2009; Pierce et al., 2004).

Separation of Growth and Proliferative Pathways

The ability to separately regulate the growth and proliferation pathways provides a simple mechanism to vary cell size and provides the flexibility needed to produce the great diversity of cell types found in the vast spectrum of multicellular organisms. Thus, in organisms as diverse as *C. elegans*, *Drosophila*, and mouse, cell growth and cell proliferation are frequently out of synchrony. This allows multiple cell divisions to occur without growth within an embryo isolated from nutrition and then to grow rapidly once nutrition is established but after the establishment of a basic body plan. Moreover, it is a simple mechanism to allow the continued growth of complex tissues during the maturation of many organisms (O'Farrell, 2004).

This does not of course mean that the proliferative and growth pathways cannot or are not frequently coupled. For example, to produce a large number of identical cells rapidly—for instance during the clonal expansion of B or T cells following an infection—it makes sense to couple the pathways (Figure 4). This need not require a cell-size-sensing mechanism, however, but only that the signals stimulating the response activate both growth and division pathways. The easiest way to achieve this is by the receptor for such a signal being wired to activate both pathways. This is clearly seen for IL-3, for example, a ligand that simultaneously activates the proliferative, growth, and survival pathways in B cells, and is a simple way to produce a large number of similar cells (Lum et al., 2005). Conversely, however, other cells need to separate these processes. Schwann cells are a good example. They migrate along axons and exit the cell cycle prior to birth. They then grow massively, concentrically

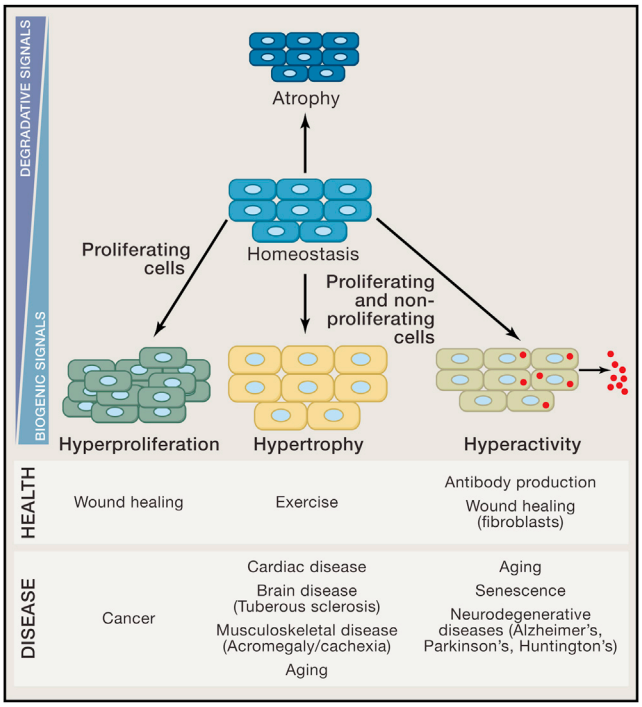

Figure 5. Loss of Homeostatic Size Control in the Adult Results in Disease and Aging

wrapping around an axon and forming a myelinating cell. However, these myelinating cells are regenerative and can re-enter the cell cycle at any moment following an injury signal; thus, these cells are quiescent rather than postmitotic. This behavior requires a clear separation of the growth and proliferative pathways to allow growth in a cell that is poised to enter the cell cycle. The separation of these pathways has been demonstrated in vitro in that distinct extracellular factors can differentially regulate the growth and proliferative pathways. Thus, whereas a growth factor such as IGF can drive growth but not proliferation, the Schwann cell mitogen NRG can drive proliferation independently of growth (Roberts and Lloyd, 2012).

Disease

The development of a tumor does not only reflect loss of a cell's normal proliferative controls but also reflects loss of normal growth and survival controls that are needed to produce and maintain the additional cell mass. All the main signaling pathways that, when mutated, are known to drive tumorigenesis have been linked to the regulation of growth control. In particular, the PI3K/AKT/mTOR pathway is activated in most tumors, for example, by activation of upstream regulators such as Ras or by the loss of negative regulators such as PTEN (Dazert and Hall, 2011). Myc is activated in many tumors and, recently, the Rb pathway has also been shown to be an important regulator of cell growth and, moreover, cooperates with oncogenic Ras/PI3K signaling to drive sustained growth (Collins et al., 2012). The realization of the universal deregulation of growth controls in tumors has led to the development of new therapeutics, which are based either on the direct targeting of the growth signaling pathways—for example, mTOR inhibitors are now showing promise in the clinic—or by targeting the metabolic pathways that are differentially activated in growing cells (Zoncu et al., 2011).

So, whereas cancer involves the loss of both cell growth and cell proliferation controls—albeit with the additional loss of tissue boundary controls that can lead to the invasion and metastasis of the expanding tumor mass—many other diseases are increasingly thought to involve the overgrowth of nonproliferating tissues. In other words, the deregulation of cell growth controls in any tissue is likely to result in disease, with too much signaling resulting in hyperactivity, hypertrophy, or cancer, whereas too little signaling will result in atrophy (Figure 5). This deregulation in cell growth controls can be caused by extrinsic or intrinsic mechanisms. For example, an increase in growth hormone signaling and hence elevated levels of IGF1 signaling in the adult result in acromegaly, whereas the deregulated growth seen in cancer is mostly driven by intrinsic genetic changes.

Cardiac hypertrophy is a major health condition that involves deregulated growth. It is commonly brought about by a diseased cardiovascular system promoting a compensatory hypertrophy of cardiac myocytes that eventually leads to aberrant contractile function. More surprisingly, perhaps, a number of developmental brain disorders are likely to be the result of deregulated growth. This first became clear when the genetic defects involved in a number of these disorders were identified and shown to be regulators of growth pathways. These include Lhermitte-Duclos disease (heterozygous *PTEN* mutations) and tuberous sclerosis (heterozygous *TSC1* or *TSC2* mutations), which both result in overactivation of the mTOR pathway. These disorders result in variable intellectual and behavioral disabilities, epilepsy, and often autism and are associated with a brain "overgrowth" pathology associated with large, dysmorphic neurons, hypertrophic astrocytes, and giant cells, as well as defects in synaptic function (Crino, 2011). In many ways, these abnormal regions of the brain can be thought of as "tumors" of nondividing cells—a point reinforced by the observation that some affected parts of the brain have lost the second tumor suppressor allele (Kwon et al., 2001). These disorders are considered developmental disorders—brain abnormalities occurring during embryogenesis—and they were considered irreversible. Remarkably, mouse genetic models that mimic the brain disorders both at the cellular and behavioral level can be largely "corrected" by treating the animals postnatally with rapamycin, an inhibitor of mTOR. Not only do these studies provoke rethinking of how these patients are regarded and might be treated, but they once more point to the dynamic nature of homeostatic cell size control. This is further exemplified by the observation that the mice need to be continually treated, as the cells regrow to their pathological size following rapamycin withdrawal (Goto et al., 2011).

Increasingly, defects in growth control and cell size homeostasis are being linked to aging (Zoncu et al., 2011). Although much of the aging process of an individual species is genetically determined, organismal lifespan can be modulated by environmental signals. A good example is the effects of dietary restriction on lifespan. In organisms as diverse as *C. elegans* and mouse, a restriction of specific components of the diet results in a

substantial increase in the longevity of the organism, at least partly due to a reduction in IGF signaling (Niccoli and Partridge, 2012). A current view is that increased biogenesis, as a result of elevated signaling through the IGF/PI3K/AKT/mTORC1 pathway, eventually leads to cellular defects mostly associated with the accumulation of damaged macromolecules (Bové et al., 2011; Zoncu et al., 2011). This has been hypothesized to result from both an overload of the biosynthetic apparatus—and an accumulation of improperly processed cell material and a defect in the degradative machinery—with an inhibition of cellular autophagy, causing the accumulation of defective cellular structures and organelles that can contribute to aging-related disorders such as Alzheimer's (Figure 5).

A recent fascinating study has also shown how other systemic signals that regulate cell size can contribute to the aging process (Loffredo et al., 2013). In mice (and humans), cardiac hypertrophy is often observed in older animals. This is associated with a substantial increase in the size of individual cardiac myocytes, resulting in an overall increase in the size of the heart. Remarkably, parabiotic association (the permanent joining of the blood-streams) of a young mouse with an old mouse resulted in the aged heart returning to its youthful size within 4 weeks. The systemic factor was identified as growth differentiation factor 11 (GDF11), a member of the TGFβ family, the levels of which drop precipitously in old animals. Similarly to myostatin in skeletal muscle, GDF11 negatively regulates cardiac muscle cell size, presumably by a similar mechanism. This study further shows how apparently stable changes in cell size associated with a pathological disorder can be corrected by re-establishing the correct balance of signals that normally set the homeostatic cell size.

Conclusions

The regulation of cell size remains mostly a mysterious process. Highly dynamic cells can maintain their size for life but can also grow or shrink, requiring robust but adaptable controls. Cell-type-specific examples are starting to give clues to the mechanisms that control cell size and highlight the complexity and diversity of the pathways involved. Moreover, the loss of these controls can lead to a variety of diseases, emphasizing the importance of these controls. As technological improvements in areas such as mass spectrometry and imaging allow us to measure the dynamic turnover of cellular components with increasing sensitivity and precision, we can look forward to exciting new insights in this important field of study.

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