

# Connections between growth and the cell cycle

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To maintain a constant size during cellular proliferation, a cell's growth rate must match its rate of division. Factors that govern proliferation must therefore coordinately regulate two distinct processes: the cellular biosynthesis that drives accumulation of mass, and progression through the cell division cycle. Recent work has identified several mechanisms which couple cell division to growth. Different mechanisms are used at different times during development to coordinate growth, cell division, and patterning.

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**Current Opinion in Cell Biology** 1998, **10**:784–790

<http://biomednet.com/elecref/0955067401000784>

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## Abbreviations

<b>CDK</b>	cyclin-dependent kinase
<b>eIF4E</b>	eukaryotic translation initiation factor 4E
<b>ERK</b>	extracellular signal related kinase
<b>PI3-K</b>	phosphatidylinositol 3-kinase
<b>Rb</b>	retinoblastoma
<b>S6K</b>	p70 S6 kinase
<b>TAF</b>	TATA box binding protein associated factor
<b>UBF</b>	upstream binding factor
<b>uORF</b>	upstream open reading frame
<b>UTR</b>	untranslated region

## Introduction

The phenomenal increase in mass which most organisms undergo during development is accompanied by a precisely coordinated increase in cell number. Such coordination of growth and cell division ensures that cells keep a fairly constant size, and that developing tissues and organs achieve the proper size and cell density. Deviations from this coordination result in unbalanced growth [1], an unstable condition which leads to abnormal cell sizes and culminates in cell death. How are the distinct processes of mass accumulation and cell cycle progression coupled? In this review, we consider four mechanisms by which cell division and growth may be linked (Figure 1). Given the multitude of factors regulating proliferation, we stress that more than one of these mechanisms may operate simultaneously. Moreover, the relationship between growth and division is influenced by environmental conditions, cell type, and developmental context.

### Mechanism one: control of growth rate by cell cycle progression

Progression through a cycle of cell division could potentially serve as a growth stimulus in a number of ways (Figure 1a). First, replication of the genome during S

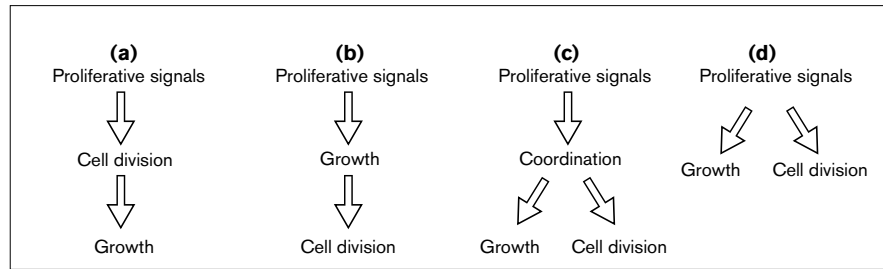
phase doubles the number of DNA templates available for transcription, thus enabling an increased rate of accumulation of RNA and protein as cells pass into the G<sub>2</sub> phase of the cycle. Indeed, measurements of dry mass or protein synthesis generally show an increased rate of accumulation about halfway through the cycle [2,3]. Second, due to the reduced size of daughter cells following mitosis, cells in G<sub>1</sub> have a large surface-to-mass ratio, and thus are potentially capable of supporting increased rates of incorporation of cellular constituents via endocytosis. Third, one can postulate the existence of an intrinsic cell size regulator that stimulates biosynthesis in response to the reduced cell size that follows mitosis.

Despite these theoretical considerations, a model whereby cell division drives growth is not well supported in the literature. For example, no acceleration of growth rate is observed in budding yeast in which cell cycle entry is advanced by overexpressing the G<sub>1</sub> cyclin Cln3. Instead, cell size is reduced [4]. Similar results are observed in vertebrate cells overexpressing the G<sub>1</sub> cyclins D1 or E [5,6,7]. Furthermore, numerous studies have shown that when cell cycle progression is specifically blocked, cell growth generally continues, resulting in abnormally large cell sizes [8–11].

Several recent reports addressing these issues in the context of a developing organism have drawn similar conclusions. Ectopic expression of cell cycle regulators in the developing *Drosophila* wing was found to alter cell cycle rates with virtually no effect on growth rates [12••]. Clones of cells constitutively expressing the *Drosophila* cell cycle activator dE2F divide at a rate twice that of cells expressing the dE2F antagonist RBF (a member of the retinoblastoma family). Despite these differences in cell doubling times, the size of the territories ultimately occupied by such cell clones is similar, regardless of cell division rate. This disparity between division and growth rates results in marked cell size changes: cells overexpressing dE2F divide faster than they grow and thus become smaller, whereas RBF overexpression causes an increase in cell size by slowing cell division without a commensurate decrease in growth. A complete block of cell division during later wing development, using a temperature-sensitive mutant allele of the mitotic cyclin dependent kinase (CDK) *cdc2*, can also uncouple growth and division [13••]. Mature wings that arise from these manipulations are of normal size and shape, but consist of fewer, larger cells. Similarly, constitutive expression of a dominant negative allele of *cdc2* during development in *Arabidopsis* results in a normal size plant comprised of fewer, but larger cells [14]. Finally, failure to appropriately exit the cell cycle is observed in *Caenorhabditis elegans* animals carrying a mutation in the *cul-1* gene, which

**Figure 1**

Four possible relationships between cell division and growth. **(a)** Cell division drives growth. **(b)** Growth drives cell division. **(c)** Growth and cell division rates are controlled in parallel by a common upstream regulator. **(d)** Independent regulation of cell division and growth.



encodes a member of the cullin family of proteins involved in proteolysis of cell cycle regulators and other proteins [15]. The extra cycles of cell division result in animals with more cells, each of which is smaller.

Thus, growth and patterning during development do not appear to be driven by increases in cell number. It should be noted, however, that slowing or stopping cell division at very early stages of wing development can eventually suppress growth, suggesting that a maximal cell size compatible with viability is reached, or that growth becomes limited by the number of DNA templates [12<sup>••</sup>,13<sup>••</sup>]. We conclude that while cell cycle progression appears to be unable to drive growth, in some cases inhibition of cell division can lead indirectly to growth suppression.

An apparent exception to this conclusion is found in the phenotype of mice carrying a disruption of the gene encoding p27<sup>(Kip1)</sup>, an inhibitor of cyclin D- and cyclin E-associated kinases [16–18]. Although normal at birth, p27<sup>(Kip1)</sup> knockout mice eventually grow substantially larger than their littermates. While this overgrowth phenotype could be an indirect effect due to enhanced endocrine signaling, serum levels of several growth stimulating hormones are unchanged. Moreover, the organs most severely affected are those which normally have the highest p27<sup>(Kip1)</sup> expression levels, suggesting that the effect of p27<sup>(Kip1)</sup> on growth is cell autonomous. These findings indicate that p27<sup>(Kip1)</sup> may possess growth-inhibiting properties in addition to its known effects on CDKs, or that failure to undergo a developmental cell cycle exit in mammals may cause cells to be refractory to growth inhibitory signals.

### Mechanism two: control of cell cycle progression by growth rate

Many types of cells govern proliferation largely during the G<sub>1</sub> phase of the cycle, at a point called ‘Start’ in yeast and the restriction point (R) in animal cells. To pass beyond this point, cells must attain a sufficient growth rate, and in particular a threshold level of protein synthesis [8,19–22]. Thus, one potential mechanism of linking cell division to growth is to couple the expression or activity of one or more cell cycle regulators to the biosynthetic rate of a cell (Figure 1b).

In budding yeast, the G<sub>1</sub> cyclin Cln3 is emerging as a primary indicator by which the cell cycle apparatus measures cellular growth rates. Cln3–Cdc28 kinase complexes initiate expression of a cascade of downstream cyclins, as well as a number of genes required for DNA replication [23,24]. Cln3 levels appear to respond to the cellular growth rate via several inputs. First, the rate of *CLN3* transcription has been shown to be modulated in response to the quality of growth medium: *CLN3* message decreases rapidly in cells grown without a fermentable carbon source, and is induced 5- to 10-fold within five minutes of addition of glucose to cells grown to post-log phase [25<sup>•</sup>]. Interestingly, the ability to undergo glycolytic metabolism appears to affect *CLN3* message levels more than the overall growth rate, as elimination of sulfur or phosphorus from the medium does not cause a decrease in *CLN3* mRNA, despite causing an efficient growth arrest.

Translational control appears to play an especially important role in regulating Cln3 expression, as modest inhibition of protein synthesis causes a disproportionate decrease in the level of Cln3 protein, and the wide range in Cln3 protein levels in cells grown on different carbon sources is greater than can be accounted for by changes in transcript levels [26,27<sup>••</sup>,28]. Removal of 5′ and 3′ untranslated regions of the *CLN3* message abolishes the induction of Cln3 expression by cAMP, which may act as a link between carbohydrate metabolism and protein synthesis [28]. A short upstream open reading frame (uORF) in the 5′ leader sequence of the *CLN3* message may account for its translational regulation in response to growth rate [27<sup>••</sup>]. Elimination of this uORF reduces the sensitivity of *CLN3* to translational inhibition, and rescues the Start delay associated with suboptimal growth conditions, such as in medium containing glycerol as the carbon source, or in a *cdc63* (a subunit of translation initiation factor 3) mutant background [27<sup>••</sup>]. A leaky scanning mechanism was proposed to account for these results, in which the translational apparatus is able to bypass the uORF during rapid growth conditions, but not when translation rates are low.

Due to the short half-life of Cln3 protein [29], such changes in its rate of synthesis rapidly affect its concentration in the

cell. Cln3 protein turnover is itself an additional target of regulation. For example, removal of nitrogen from the growth media has been found to both increase the rate of ubiquitin-mediated Cln3 degradation and decrease the Cln3 translation rate [30]. Thus, the concentration of Cln3 protein is linked to cellular biosynthesis rates by a variety of mechanisms, providing a means of precisely tethering the timing of Start entry to the rate of cell growth.

Do the levels or activities of cell cycle regulators respond to growth rates in similar ways in animal cells? Metazoan cell division has evolved in an environment somewhat buffered from external nutrient conditions, and is regulated primarily through hormonal control and cell–cell interactions. Nonetheless, limited evidence suggests that some of the above mechanisms potentially could be used to couple cell cycle regulators to cell growth rate. For example, several cell cycle proteins have either been shown to be translationally controlled, or have mRNAs with complicated 5' leader sequences suggestive of such regulation. These include CDK4 and its partner cyclin D1 (whose expression and activity in higher eukaryotes are similar to that of Cln3 in yeast), the CDK inhibitor p27<sup>(Kip1)</sup>, and the oncoprotein Mdm2 [31–35]. Therefore, in the same way as for Cln3, efficient expression of these proteins may be restricted to conditions favoring maximal growth and protein synthesis. It has not yet been shown, however, whether such regulation is actually used to couple cell cycle progression to cellular metabolic rates.

### Mechanism three: coordinate control of growth and cell division

Regulation of biosynthesis and cell cycle progression by a common signaling pathway provides another powerful means of pairing these processes (Figure 1c). Factors that act at the branchpoints of such pathways can potentially serve to coordinate growth and division. Several factors that may act as such coordinate regulators are discussed below.

#### TATA box binding protein associated factors

The yeast transcriptional coactivator  $\gamma$ TAF(II)145 appears to be one such factor that could act at the branchpoint of a common signaling pathway for growth and cell division. Like other TATA box binding protein associated factors (TAFs),  $\gamma$ TAF(II)145 is required for activated transcription of a specific subset of genes. It was recently found that the targets of  $\gamma$ TAF(II)145 include numerous genes that are required for cell cycle progression, including G<sub>1</sub> and S phase cyclins [36•], as well as growth-related genes such as those encoding ribosomal proteins [37•]. Expression of  $\gamma$ TAF(II)145 itself is responsive to cellular growth state, becoming reduced as cells are grown to high density [36•]. These findings are consistent with a mechanism whereby conditions favorable for rapid growth lead to induction of  $\gamma$ TAF(II)145, which then directs a coordinate transcriptional program of genes required for biosynthesis and cell cycle advancement. Since inactivation of  $\gamma$ TAF(II)145 causes a

G<sub>1</sub> arrest similar to the mutant phenotype of its mammalian homolog, TAF(II)250 [38,39], such a mechanism of transcriptional co-regulation of growth-related genes and cell cycle genes may be evolutionarily conserved.

#### Ras

Ras activation leads to the upregulation of a number of Ras effector networks, including the mitogen-activated protein (MAP) kinase and phosphatidylinositol 3-kinase (PI3-K) signaling pathways [40]. Two targets of these pathways, the eukaryotic translation initiation factor 4E (eIF4E) and the p70 S6 kinase (S6K), are potential modulators of cell growth via their effects on protein synthesis. It has been shown that eIF4E binds to the 5' cap of all mRNAs and may have an especially important role in regulating translation of messages whose leader sequences contain complicated secondary structure [41•]. Phosphorylation of eIF4E correlates with increased translation rates. Recent work shows that eIF4E phosphorylation is regulated by multiple signaling pathways, since inhibitors of either extracellular signal related kinase (ERK) or p38 MAP kinase can block eIF4E phosphorylation in response to a variety of stimuli [42,43]. The pathways appear to converge on a novel protein kinase, Mnk1, which is able to phosphorylate eIF4E *in vitro* [44•,45]. eIF4E activity is also regulated by the inhibitory binding protein 4E-BP1, which is a target of the PI3-K pathway. Activation of PI3-K leads to signaling through the Akt and mTOR kinases, and results in the phosphorylation of 4E-BP1 and its release from eIF4E [46,47]. PI3-K/Akt/mTOR signaling also influences protein synthesis rates by activating S6K [48], which phosphorylates the 40S ribosomal subunit S6 and leads to increased translation of ribosomal protein mRNAs [49].

This potential upregulation of cellular biosynthesis by Ras may be balanced by a direct stimulatory effect on cell-cycle progression. Expression of a dominant-negative Ras mutant Ras(Ser17→Asn) blocks multiple responses of quiescent cells to serum stimulation, including induction of cyclin D1, degradation of the CDK inhibitor p27<sup>(Kip1)</sup>, activation of E2F target genes, and entry into S phase [50•–53•]. Since Ras(Ser17→Asn) expression does not block S phase entry in cells lacking the retinoblastoma (Rb) protein, nor in cells that have passed the restriction point, the major cell cycle function of Ras in these cells appears to be to inactivate Rb during late G<sub>1</sub>. It is important to note, however, that it is not yet clear whether these cell cycle effects are due directly to Ras activation, or whether they are secondary consequences of the stimulatory effects of Ras on metabolic rate.

#### Myc

Expression of the Myc transcription factor is associated with proliferation in many cell types [54•]. Myc has been proposed to stimulate progression into S phase by several distinct mechanisms: direct transcriptional activation of the CDK-activating phosphatase CDC25A [55], inactivation or sequestration of p27<sup>(Kip1)</sup> [56,57], and upregulation

of *cyclin E* expression [57,58]. A hormone-inducible system (MycERTM) was recently used to test the effect of myc expression in exponentially growing rat fibroblasts [59]. Although Myc induction caused premature activation of cyclin E-, cyclin A- and cyclin D1-associated kinases, entry into S phase still did not occur until a normal cell size was reached, and the overall rate of cell division was unchanged. These results imply that myc can't override a size control on S phase entry, and is unable to accelerate the growth rate of already rapidly growing cells, at least in this transformed cell line. Nonetheless, a number of genes implicated in growth control are activated by myc, including *elF4E* [60], *elF2 alpha* [60], *BN51* (an RNA polymerase III subunit) [54\*], *nucleolin* (involved in ribosome biogenesis) [54\*], *ornithine decarboxylase* (polyamine biosynthesis) [61] and *CAD* (pyrimidine biosynthesis) [62]. Surprisingly, myc function does not appear to be essential for proliferation, as rat fibroblast cell lines with targeted disruptions in both copies of the *c-myc* gene (and with no detectable expression of N-*myc* and L-*myc*) are viable, albeit extremely slow growing [63\*]. Rates of RNA and protein synthesis are reduced in *c-myc* null cells, and both G<sub>1</sub> and G<sub>2</sub> phases of the cell cycle are extended. Importantly, these reductions in growth and proliferation rates occur in synchrony, and as a result cell size and mass are unchanged from that of parental control cells.

### Retinoblastoma

The ability of tumor suppressors such as Rb and p53 to inhibit proliferation in a balanced manner is consistent with these proteins acting as a check on both cell cycle progression and growth rates. Although Rb has been referred to as a 'growth suppressor' this term is often applied loosely, usually in reference to its well characterized effects on cell cycle regulation. Nonetheless, our understanding of the roles these proteins may play in regulating cellular growth is increasing. The observation that Rb<sup>-/-</sup> fibroblasts have a reduced sensitivity to cycloheximide [64] suggests that Rb might suppress growth in part by inhibiting protein synthesis. Interactions between Rb and each of the RNA polymerases I, II, and III may be involved in this regulation. Rb has been shown to repress RNA polymerase I-mediated transcription of 45S rRNA *in vitro*, by binding to the RNA polymerase I transcription factor UBF [65], and inhibiting its DNA binding activity [66]. Rb is also a potent inhibitor of RNA polymerase III [67], which synthesizes numerous small stable RNAs required for protein synthesis and thus for growth. RNA polymerase III transcription can be repressed by transient transfection of Rb as well as in a cell-free system, and cells lacking functional Rb have an approximately fivefold increase in RNA polymerase III activity. As in the case of RNA polymerase I, Rb appears to inhibit RNA polymerase III by binding to and inactivating a general accessory factor required for transcription, in this case TFIIB [68,69\*]. Interestingly, Rb contains regions of sequence related to two subunits of TFIIB, and thus may interfere with TFIIB assembly

or activity by mimicking or displacing these subunits [69\*]. Finally, Rb has also been shown to bind directly to the RNA polymerase II coactivator TAF(II)250 [70,71], which by analogy with its yeast counterpart yTAF(II)145 may target specific growth-related genes such as ribosomal protein subunits, as described above. Thus, in addition to its canonical role in repressing transcription of the E2F target genes required for promoting DNA synthesis, Rb may effect a simultaneous downregulation of growth via a multifaceted inhibition of ribosome biogenesis. Whether any of these interactions, however, actually limit growth *in vivo* is not yet known and it remains possible that growth suppression by Rb is primarily due to its cell cycle effects, as shown for the *Drosophila* Rb homolog, RBF [12\*\*].

### p53

A similar role in repressing cellular biosynthesis has recently been proposed for p53. DNA damage and other genotoxic stresses induce a cell cycle withdrawal which is mediated largely by p53-dependent transcriptional activation of cell cycle inhibitors [72,73]. For cells to remain viable during such periods of cell cycle inactivity, biosynthesis rates must also be downregulated. In such cases, p53 may suppress growth by inhibiting the transcriptional activity of RNA polymerase III [74,75\*]. Overexpression of p53 reduces transcription of RNA polymerase III templates *in vitro* and in transfected cells, and extracts from cells lacking p53 have up to 25-fold higher rates of RNA polymerase III activity. As in the case of Rb, p53 appears to regulate RNA polymerase III activity by binding and inactivating TFIIB. It may also control translation directly, as it has been found associated with ribosomes via covalent attachment to 5.8S rRNA in some cells [76,77]. Finally, the activity of p53 itself appears to be coupled to cellular growth conditions by a DNA damage-independent mechanism, in which depletion of ribonucleotide pools causes a p53-dependent reversible arrest [78].

### Mechanism four: independent regulation of growth and division

From the foregoing discussion, it should be clear that there are many potential mechanisms to couple growth and cell cycle progression. It may also be instructive, however, to consider cases in which growth and division are regulated independently (Figure 1d). Exceptions to the rule of growth and divisional coupling are plentiful during development, such as the continued growth of many differentiated postmitotic cells, and the cleavage divisions which divide large embryos into progressively smaller cells.

During *Drosophila* development, cell cycle regulation passes through multiple phases of control, both growth coupled and uncoupled. The embryonic cell cycles prior to hatching are growth independent, and instead rely on materials loaded into the oocyte. Changes in cell cycle control

strategies during this time reflect the successive exhaustion of these maternally supplied components (reviewed in [79]). The first thirteen cycles are extremely rapid, reflecting the abundance of such maternal factors. The mitotic inducer String (CDC25) is the first regulatory component to be depleted, and as a result the next few cycles are regulated at the G<sub>2</sub>/M transition, in response to patterned zygotic expression of String. Cell size decreases with each division during this period, and the G<sub>1</sub> phases that are usually used in growth regulation are absent from the cycle. Finally, most cells in the embryo enter a quiescent G<sub>1</sub> phase upon depletion of maternal Cyclin E and the concomitant induction of the CDK inhibitor Dacapo. Interestingly, the zygotic expression of String, Cyclin E, and Dacapo appears to be controlled by patterning cues, rather than coupled to mass increase.

The subsequent larval stage is characterized by massive growth. In tissues such as the imaginal discs, cell division is coupled to increases in mass, and cell size remains fairly constant from one division to the next. These rapid larval cell cycles have no obvious spatial pattern, although apparently randomly grouped clusters of cells progress through phases of the cell cycle in synchrony [80]. As growth slows and finally stops at the end of larval development, patterning systems again take control of the cell cycle, manifested first as a synchronous cell cycle exit in discrete regions of the developing eye and wing [81,82].

Thus, control over the cell cycle appears to switch several times during fly development. Embryonic and imaginal patterning systems control division during times of no or little growth, allowing coordination of the cycle with morphogenetic movements and differentiation. During the larval stage, when rapid accumulation of mass is paramount, the patterned, growth-independent cycles of the embryo give way to apparently unpatterned cell cycles that respond to nutritional or hormonal cues [83]. An interesting area of future work will be to dissect the mechanisms underlying the developmental switches between growth coupled and growth uncoupled cycles.

## Conclusion

The past several years have seen a tremendous increase in our understanding of how cell proliferation is regulated. Many of these gains have centered on the workings of the core cell cycle machinery, and the signaling pathways that control it. A current gap in our knowledge lies in understanding how these pathways regulate growth itself, particularly in the context of an intact tissue, where multiple modes of regulation are possible. Since growth control appears to be dominant to cell cycle control in most situations, efforts aimed at learning how growth is regulated are essential for a complete understanding of cell proliferation. Gains should also be made by further inquiries into the mechanisms connecting growth and division, the relationships between these mechanisms, and how different mechanisms are used for distinct developmental purposes.

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