Coordination of cell growth with cell division
Michael Polymenis*† and Emmett V Schmidt**

Proliferating cells must increase their mass coordinately with cell division. Recent evidence suggests that coupling of cell growth with cell division might be achieved by making synthesis of activators of cell division particularly sensitive to the capacity of the cell’s protein synthesis machinery.

Addresses
*MGH Cancer Center, Massachusetts General Hospital Building 149, 13th Street, Charlestown, Massachusetts 02129, USA
†The Pediatric Service, Massachusetts General Hospital and the Department of Pediatrics, Harvard Medical School, Fruit Street, Boston, Massachusetts 02114, USA;
e-mail: schmidt@helix.mgh.harvard.edu
‡e-mail: polymeni@helix.mgh.harvard.edu
Correspondence: Emmett V Schmidt

http://biomednet.com/elecref/0959437X00900076
© Elsevier Science Ltd ISSN 0959-437X

Abbreviations
Cdk cyclin-dependent kinase
uORF upstream open reading frame

Introduction: coordination of cell growth and division during the G1 phase of the cell cycle

Overall cellular parameters, such as cell size and macromolecular composition, remain remarkably constant in continuously dividing cells for any given rate of cell proliferation (Figure 1). This fundamental phenomenon results from tight coordination between cell growth and cell division. How the cell manages to coordinate its division, which is marked by discrete morphological and molecular changes, with the continuous accumulation of macromolecular components is a question that has puzzled biologists for decades.

Variability in cell proliferation rates correlate with the time spent in the first gap phase (G1) of the cell cycle. As rates of cell proliferation decrease, doubling times increase because the G1 phase is prolonged. Growth limitation by removing nutrients, growth factors or inhibiting protein synthesis usually causes a G1 arrest. This has led to the concept that G1-specific events coordinate cell growth with division. Completion of these events presumably allows cells to pass through some transition — termed START in yeast or the restriction point in mammalian cells — which triggers cells to initiate DNA replication, committing them to division [1–3].

What is the nature of the trigger that allows cells to complete START? Early studies suggested that levels of a very unstable protein might serve as a trigger because certain levels of inhibition of protein synthesis specifically limit passage through the restriction point [1]. Although an enhanced cell-growth requirement undoubtedly exists in G1, these findings do not necessarily imply that the demand for cell growth is limited to the G1 phase. Cell growth is continuous after G1 and ongoing protein synthesis is necessary for completion of later events of the cell cycle [3,4]. For example, different mutations in a single translation initiation factor in Saccharomyces cerevisiae have varying effects, with the most severe alleles affecting completion of all phases of the cell cycle whereas weaker ones preferentially affect G1 [5].

The identity of the labile protein(s), which mediate passage through START, has remained elusive. Attention, however, has focused on the G1 cyclin proteins — the activating regulatory subunits of cyclin/cyclin-dependent kinase (Cdk) complexes.

Cell-growth controls that regulate G1 cyclin/Cdk complexes and cell division

Altering the levels of most G1 cyclins alters the timing of START in yeast and other eukaryotes, suggesting that these proteins are rate limiting for the G1/S transition. In mammalian cells, a variety of mitogenic signals converge on the regulation of the activity of G1 cyclin/Cdk complexes [2]. It is also clear that mitogenic stimulation of protein synthesis overall is necessary for sustained cell proliferation [6]. The importance of G1 cyclin/Cdk activities in mediating mitogenic effects was illustrated in a recent study showing that activated G1 cyclin/Cdk complexes were sufficient for initiation of DNA replication in quiescent human cells, in the absence of any mitogenic signals [7*].

Cln3p is a G1 cyclin in S. cerevisiae that functions upstream of all other cyclin/Cdk regulated events; it activates the START transcriptional program including transcription of other G1 cyclins [8]. The timing of its function makes Cln3p an attractive candidate for playing a critical role in coupling cell growth with division. Cln3p is extremely unstable and its levels do not vary much during the cell cycle, therefore the rate of Cln3p synthesis probably determines its overall abundance.

How might cell growth regulate Cln3p levels? We recently described a short upstream open reading frame (uORF) in the S’ leader of the CLN3 mRNA which lowers the translational efficiency of the CLN3 message [9**]. In agreement with a model originally proposed by Harvey Lodish for mRNAs of low translational efficiency [10], the CLN3 mRNA is translated well when the ribosome content of the cell is high but it is disproportionately inhibited as the cell’s ribosome content decreases [9**]. Thus, Cln3p synthesis is very sensitive to the overall levels of active ribosomes within the cell. As the cellular ribosome content decreases as growth rate slows, this mechanism ensures that Cln3p will not accumulate to a certain threshold level and DNA replication will not be initiated in poor growth conditions. The translational repression in Cln3p synthesis is relieved, however, when the ribosome content is high,
Cell growth refers to increases in macromolecules exclusive of DNA. Cell division refers to the doubling of DNA, which is coupled to mitosis once DNA synthesis begins. Cell proliferation is the increase in number of cells within a tissue or culture. Obviously, the total number of cells is determined not only by the rate they are generated but also by the rate cells are dying. (For simplicity, aspects of cell death will not be considered here.) On the basis of experiments performed in budding yeast, Drosophila and mammalian systems, in our review we describe mechanisms by which regulatory molecules might affect cell division through their control of (a) cell growth. We also discuss factors that (b) regulate both processes simultaneously. Finally, we describe situations in which regulatory molecules might limit cell proliferation through (c) their control of the cell cycle.

Yeast and other eukaryotic daughter cells do not divide until they reach the size of their mothers, and they have a longer G1 phase [3]. Assuming that the concentration and sub-cellular localization of cellular components is the same in both large and small cells, this 'cell size control' is thought to reflect the need to accumulate a given absolute amount of a key activator of cell division [3]. There are, however, at least two additional scenarios (see also [15**] for an interesting discussion of the issue): first, the well-established unequal distribution of certain factors during cell division could very well affect subsequent cell cycles. Recent evidence suggests that this might be the case for Ash1p, a budding yeast transcriptional repressor that is specifically localized to the nucleus of the daughter cell (M Maxon, I Herskowitz, personal communication). Second, it is possible that the critical size requirement for division does not reflect the accumulation of an activator of cell division but rather the dilution of an inhibitor. Several studies have implicated p27kip1, a mammalian Cdk inhibitor, in such a role.

The levels of p27kip1 alter the response to mitogenic signaling and they are primarily regulated by post-transcriptional mechanisms, including translational control [16,17]. Elevated levels of Cdk inhibitors often correlate with cellular differentiation. In many tissues of complex organisms, cells divide a certain number of times prior to terminal differentiation and this determines the overall size of the animal which is predominantly a function of total cell numbers [18]. At least in oligodendrocytes, this mitogen-dependent intrinsic 'timing' process is affected by cell size and p27kip1 levels [19-22]. These studies confirmed that cell cycle times oscillated between long and short intervals in this system depending on the amount of cell mass carried from the previous cycle [20]. Importantly, p27kip1 null precursor cells undergo an additional one or two divisions before they differentiate into oligodendrocytes, which
might explain the gigantism and the higher number of cells found in a variety of organs in p27Kip1 null mice [23**]. Similarly, mice which lack the Cdk inhibitor p18INK4c also display additional cell divisions in a variety of tissues [24*]. It is interesting to note that p18INK4c levels, as with p27Kip1, are subject to translational control [25].

Early experiments in yeast demonstrated that mutations that affect cell growth also block cell division — but not vice versa [3]. The importance of cell-growth regulation was further suggested by perturbation experiments of translation factors in mammalian cells. Most dramatically, overexpression of the mRNA cap binding protein (eIF-4E) leads to cell transformation [26]. This factor is rate-limiting for translation initiation because it is the least abundant of the translation initiation factors. Perturbations of additional regulators of translation rates, such as the eIF2 activity, are similarly oncogenic [27]. Interestingly, cell size control in cells overexpressing these translation factors has received scant attention. Besides Cln3p and the Cdk inhibitors mentioned above, examples of translational control among cell-division regulators include c-myc [28], cyclin D1 [29], Cdk4 [30], and the yeast G1 cyclin Cln2p [31]. As was the case for Cln3p, identification of the regulatory elements mediating these controls might reveal intriguing physiological consequences regarding the coupling of cell growth with cell division.

Regulators which coordinate the cell cycle

Molecules that control cell proliferation through combined effects on both cell growth and cell division might be expected to have particularly potent effects on overall proliferation. Studies of the functions of c-myc target genes [32] and the recent identification of Drosophila myc [33] identify it as a regulatory molecule with effects on both cell growth and cell division. The dMyc protein is encoded by the Drosophila gene diminutive, a mutation in which results in small body size as a result of decreases in cell size. Immunoprecipitation of Myc-bound chromatin originally identified a DEAD-box RNA helicase as one key myc target gene in mammalian cells [34]. This helicase has been found to correspond to the pitchoune gene in Drosophila [35*]. The potential function of these helicases can be extrapolated from studies in yeast where twenty-six DEAD-box helicases are known [36,37]. All of the RNA helicases studied to date function either to regulate translation initiation or are needed in the cleavage steps required to process ribosomal RNA. In addition, we have shown that the translation factor eIF-4E is upregulated by c-myc [38–40]. Indeed, after Myc induction, increased protein synthesis preceded RNA synthesis by 14 hours [41]. C-myc target genes involved in cell-cycle regulation include both E2F [42] and the Cdc25 phosphatase [43] responsible for cyclin activation. C-myc further increases expression of the ada and ornithine decarboxylase gene products which are both necessary components of the DNA synthetic apparatus [32]. Similarly, Tafl145p, a yeast transcription factor specifically required for G1/S progression, regulates transcription of both G1 cyclins and ribosomal protein genes [44*,45*]. These dual effects on both cell growth and cell division provide an elegant mechanism that might ensure a balance between the two processes.

Both the product of the retinoblastoma gene (pRb) and the p53 tumor suppressor can similarly affect expression of genes involved in both growth regulation and cell division. In addition to their well known effects on cell-cycle control, both pRb and p53 inhibit RNA transcription [46,47]. Although direct effects on ribosomal assembly or protein synthesis have not been determined in these initial studies, it would be surprising to find minimal effect on these measures of cell growth. Of note, Rb-deficient cells are less sensitive to the effects of cycloheximide [48], supporting a role in restriction point control.

Are division controls rate-limiting for cell proliferation?

Over-expression of G1 cyclins shortens G1 without affecting the total inter-division time because the length of subsequent phases of the cell cycle increase in compensation. It is possible that these compensations are as a result of growth limitations imposed by controls in G2 or M phase. These observations are in apparent agreement with the early experiments where it was shown that cell growth is necessary for cell division and not the other way around [3]. Therefore, only activities involved in cell growth — and not activities involved in cell division — are expected to determine the overall rate of cell proliferation.

This subsidiary role of cell division control is elegantly demonstrated in the developing Drosophila wing [49**].
First, removal of the string phosphatase which activates mitotic Cdk activity arrested cell division but did not arrest cell growth. As a result, string-deficient cells continued to grow to larger sizes. In contrast, ectopic expression of the transcription factor dE2F, which promotes the G1/S transition, in specific compartments of the developing wing resulted in a dramatic increase in cell number per unit area. Remarkably, these experiments demonstrate that over-expression of cell division activities can determine the rates of cell proliferation, in this case in developing tissues where growth factors may be limited. Interestingly, the overall wing size was unchanged in these Drosophila experiments, in contrast to the enlarged organs of the p27\textsuperscript{kip1} and p18\textsuperscript{NKK} null mice. The boundaries of the tissue compartment were fixed and seemed to limit the overall growth of the wing which consequently contained an increased number of small cells.

**Conclusions**

The evidence we have reviewed here enhances our understanding of mechanisms by which processes involved in cell growth, such as protein synthesis, may be integrated with the machinery that brings about cell division. However, this evidence also underscores areas which need additional investigation. Importantly, neither Cln3p nor p27\textsuperscript{kip1} are essential for control of cell growth and division. Although their role is significant, they are probably one part of a more complex picture. This is not surprising, as input from many different aspects of cellular physiology could potentially affect the cell's decision to divide. The extensive research on the coordination of cell growth with division in prokaryotes, where most of the concepts discussed here were originally formulated [50], suggests that the road ahead will be long and exciting.

**Acknowledgements**

We would like to thank members of the MGH Cancer Center for helpful discussions. We especially thank Mary Mason for comments on the manuscript and communicating results prior to publication. Work in the authors’ laboratory was supported by PHS grants RO1-CA63117 and RO1-CA68069 from the NIH to Emmett Schmidt.

**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


15. Hall DD, Markwardt DD, Panviz F, Heideman W: Regulation of the Cln3-Cdc28 kinase by cAMP in Saccharomyces cerevisiae. EMBO J 1998, 17:4370-4379. Demonstrates that much of cAMP's effects on G1/S progression can be accounted for by its effects on ribosomal biogenesis and CLN3 translation because translational de-repression of the CLN3 message was sufficient to overcome the G1 arrest upon cAMP withdrawal.


23. Durand B, Fero ML, Roberts JM, Raff MC: p27kip1 alters the response of cells to mitogen and is part of a cell-intrinsic timer that arrests the cell cycle and initiates differentiation. Curr Biol 1998, 8:431-440. Using oligodendrocytes that lack p27kip1, this study establishes the role of p27kip1 in determining the number of cell divisions prior to differentiation, in response to mitogens.


44. Walker SS, Shen W-C, Reese JC, Apone LM, Green MR: Yeast TAF145 required for transcription of G1/S cyclin genes and regulated by the cellular growth state. Cell 1997, 90:807-814. This and the following paper [45] demonstrate that loss of Taf145p causes an inability to pass through START because Taf145p regulates both cell division and cell growth, as it specifically affects transcription of G1 cyclins and ribosomal proteins, respectively.


49. Neufeld TP, de la Cruz AFA, Johnston LA, Edgar BA: Coordination of growth and cell division in the Drosophila wing. Cell 1998, 93:1183-1193. This paper confirms that cell growth is a prerequisite for cell division in vivo in animal tissues but overexpression of dezf alone (or cyclin E plus string) shortened the doubling time, implying that besides regulators of biosynthetic capacity activators of cell division can also determine the overall rate of cell proliferation.