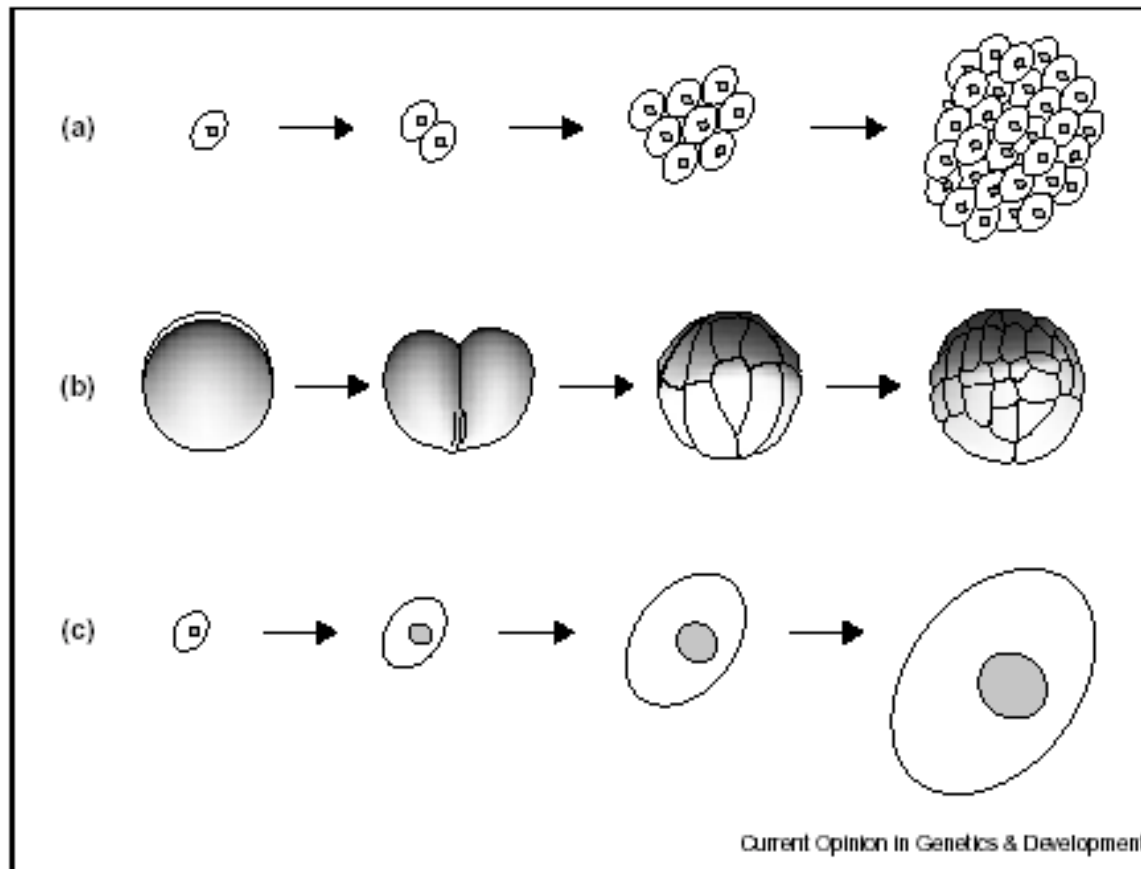
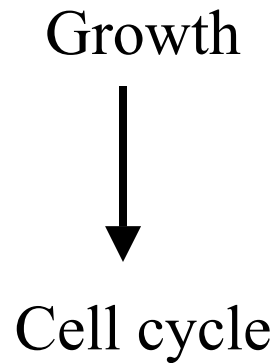


CONTROL OF CELL GROWTH



Examples of separation of cell growth and division found in nature. Whereas (a) mitotic cells often coordinate growth with division (b) early embryogenesis involves division without growth and (c) endoreduplication constitutes growth without division.

Cell size regulate proliferation!



Amoeba experiment: repeated cytoplasmic amputation prevented cell division for 6 months , while control divided 65 times.

What would happen if cell growth is not coordinated with cell division

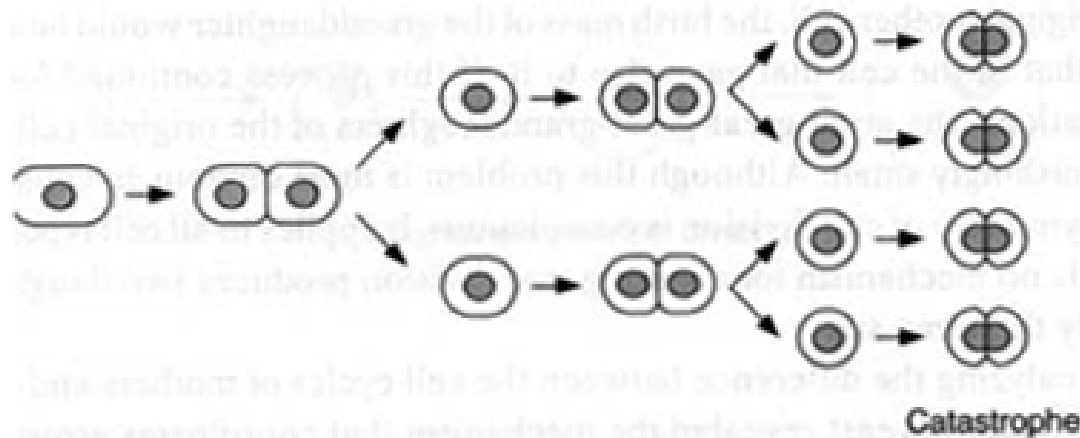


FIGURE 3-11 Coordinating cell growth and division. Cells that can complete a cell cycle in less time than it takes to double in size become progressively smaller with each generation, until they reach a size at which division kills the cells.

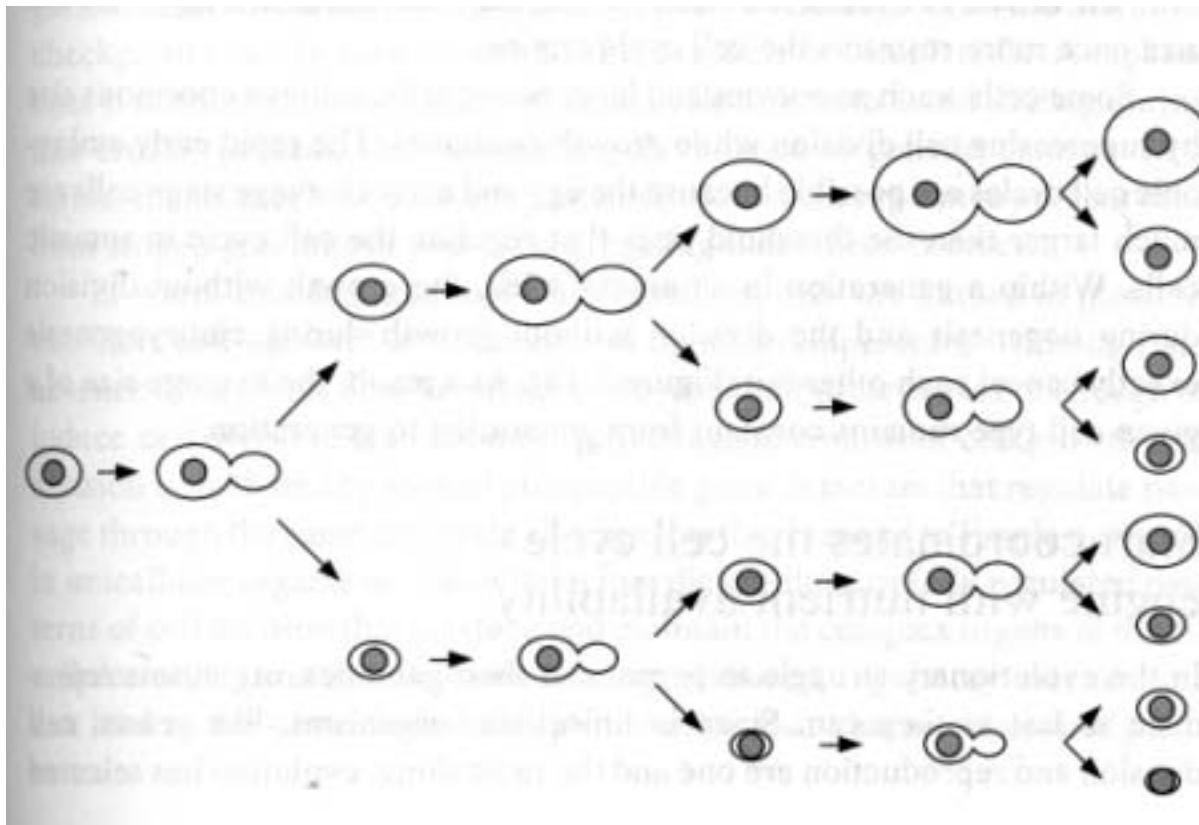


FIGURE 3-12 Problem of unequal cell division. This diagram shows what happens if cells obeying the simple rule of exactly doubling their birth mass before dividing divide unequally to produce one cell that is bigger and one that is smaller than the average birth size. To produce one average-size granddaughter, the small daughter must divide unequally, making the other granddaughter very small. The opposite is true for the large daughter, which can produce a normal-size granddaughter only at the expense of producing a very large granddaughter. Thus, the simple mass-doubling rule soon produces a population that contains very large and very small cells.

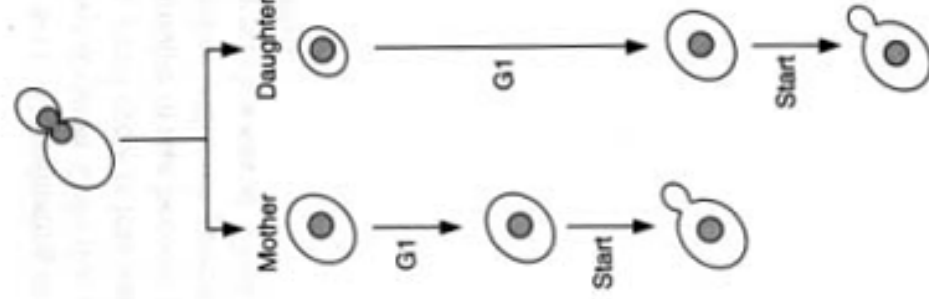


FIGURE 3-13 Threshold size for Start. The daughter cells of budding yeast, which are born smaller than their mothers, must grow more than their mothers to reach the minimum size required to pass Start. The interval between birth and Start is therefore much longer in daughters than it is in mothers. Reference: Hartwell, L. H., & Unger, M. W. *J. Cell. Biol.* **75**, 422–435 (1977).

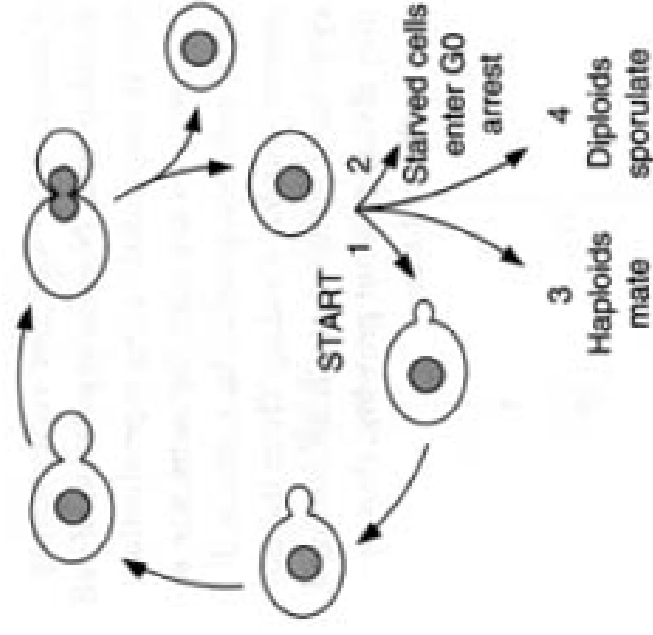
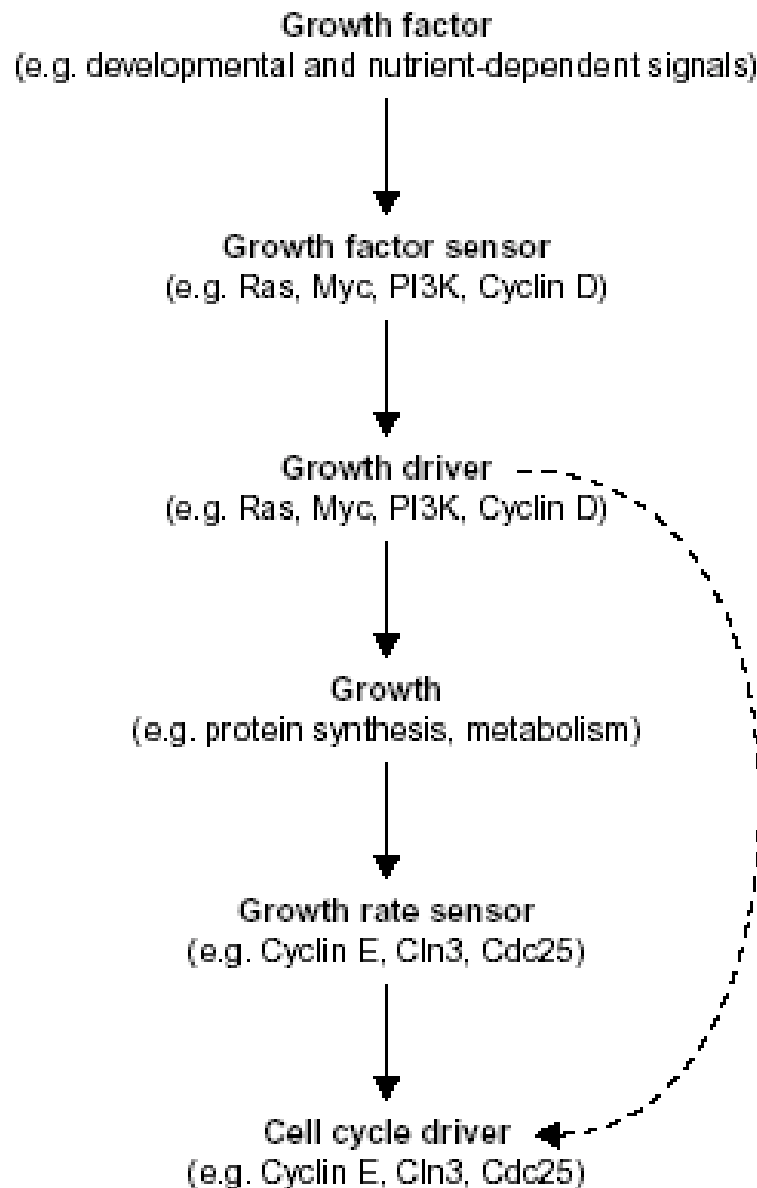


FIGURE 3-15 Alternative cell fates in budding yeast. After mitosis ends, budding yeast cells can do one of the following: (1) pass through Start, replicate their DNA, and undergo mitosis; (2) enter the resting G0 phase in response to starvation; (3) mate if haploid; (4) undergo meiosis and sporulate if diploid. Once cells have passed through Start, however, they are committed to the mitotic cell cycle and must exit mitosis before these other options are again possible.

Growth is above the cell cycle



Hierarchy of growth control. Growth factors such as developmental and nutrient-dependent signals activate growth factor sensors such as Ras, Myc, PI3K and Cyclin D. These same proteins also drive growth by increasing rates of protein synthesis and by regulating other metabolic processes. Some growth drivers may also regulate the cell cycle via growth-independent mechanisms (dashed arrow). Growth-rate sensors, such as the G_1/S limiters Cyclin E and Cln3, and the G_2/M limiter Cdc25, sense cellular growth rates and drive cell-cycle progression. Combining the tasks of sensing growth factors and driving growth, or sensing growth rates and driving the cell cycle, ensures that these processes remain coupled.

Cell division mutants (CDC)

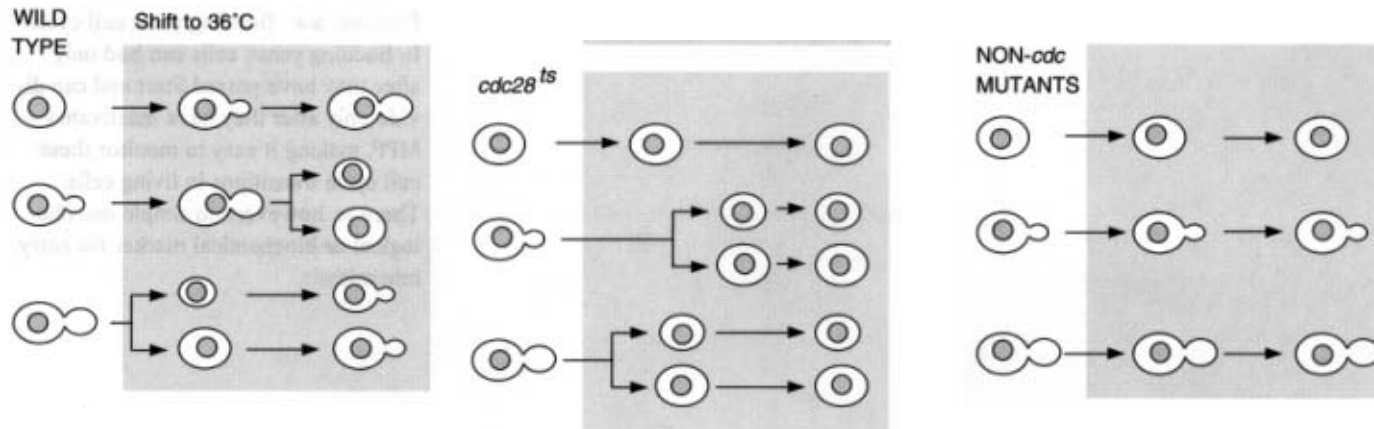


FIGURE 3-5 Cell division cycle mutants. Temperature-sensitive *cdc* mutants are distinguished from other temperature-sensitive mutants by their behavior at the restrictive temperature. Most temperature-sensitive mutations arrest cells at random positions throughout the cell cycle when an asynchronous population of cells is shifted to the restrictive temperature. A *cdc* mutant, however, arrests all cells in the population at the same point in the cell cycle. Reference: Hartwell, L. H., Culotti, J., & Reid, B. *Proc. Natl. Acad. Sci. USA* 66, 352–359 (1970).

Cell growth mutants (wee, WHI, LGE)

Genotype	Size
Wild type	
<i>CLN3-1^D</i>	
4X <i>CLN3</i>	
Δ <i>cln3</i>	

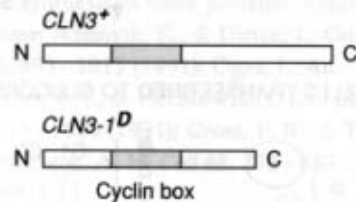


FIGURE 6-2 Cln3 is a G1 cyclin. Mutations in the *CLN3* gene affect cell size and sensitivity to α factor. The lower part of the figure shows the structure of the Cln3 protein and the carboxyl-terminal deletion that increases the stability of the protein. The cyclin box is the region of the protein that is homologous to other cyclins. Reference: Cross, F. R. *Mol. Cell. Biol.* 8, 4675–4684 (1988).

CLN3 mRNA level is constant during cell cycle

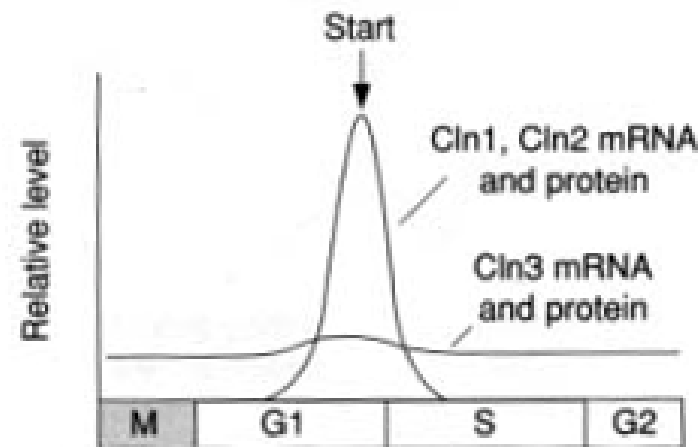


FIGURE 6-4 Regulation of G1 cyclins. The *CLN1* and *CLN2* genes are transcribed only during G1, so their protein products are present only in this part of the cell cycle. The *CLN3* gene is transcribed throughout the cell cycle, so its product is always present. The activity of the Cln3 protein is regulated by post-translational modification. Reference: Wittenberg, C., Sugimoto, K., & Reed, S. I. *Cell* 62, 225–237 (1990).

An uORF influences CLN3 translation efficiency

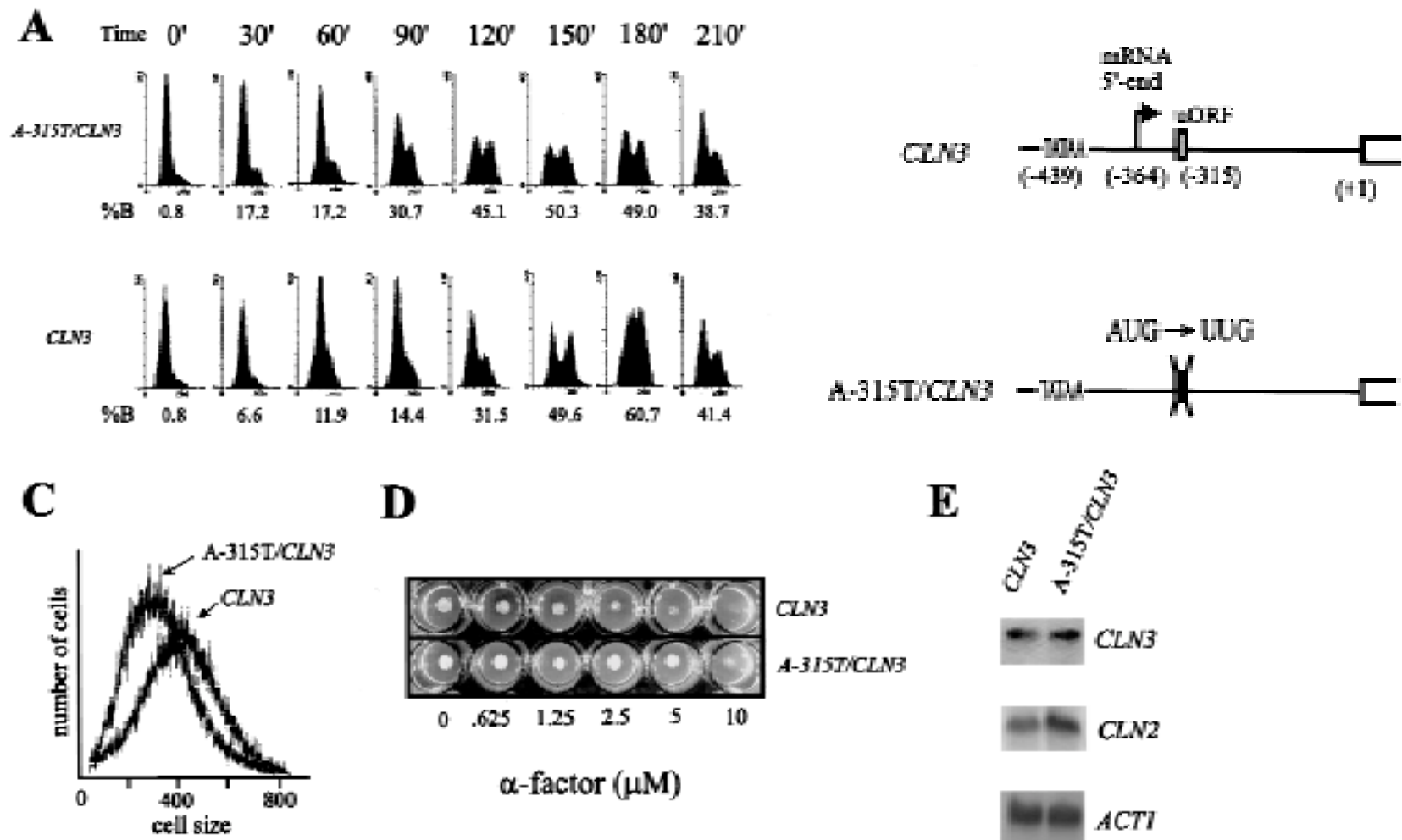


Figure 3. Inactivation of the uORF in the *CLN3* mRNA accelerates budding and DNA replication, decreases cell size at Start, and increases resistance to α -factor in poor growth conditions. (A) Small G_1 mutant and control cells were obtained by centrifuga elutriation from cultures growing in YPG to a cell density of 1×10^6 cells/ml, and resuspended at the same cell density in the original clarified medium. At the indicated time points after resuspension, the percent of budded cells (%B) and cellular DNA content was determined. (B) The cellular DNA content of mutant and control cells was determined from cultures growing asynchronously in YPG to a density of 1×10^6 cells/ml. (C) Cell size measurements of the whole cell populations grown in YPG to a density of 1×10^6 cells/ml were determined by FACS. Cell numbers are plotted on the y-axis and the x-axis represents the forward angle scattering. Cell size measurements are relative and not absolute. (D) Sensitivity to α -factor of cells growing on YPG solid medium was tested at the indicated concentrations of α -factor. (E) RNA blots of *CLN3*, *CLN2*, and *ACT1* mRNAs, as in Fig. 2D from cells grown in YPG to density of 1×10^6 cells/ml.

Growth and cell cycle are coordinated by CLN3 G1 cyclin translation

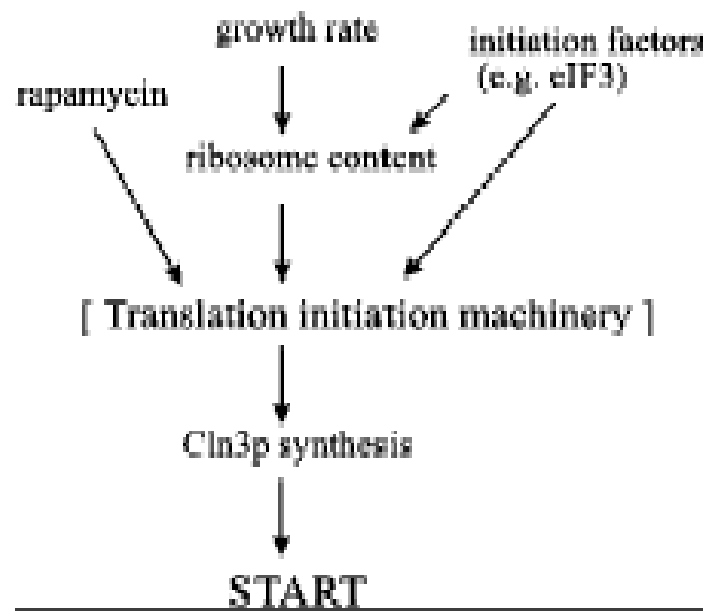


Figure 7. Cln3p synthesis, and completion of Start, are particularly responsive to changes in the concentration of the translation initiation machinery. *CLN3* expression is growth-rate dependent, because growth rate correlates with the cell's ribosome content and, therefore, with the concentration of ribosomes competent to initiate translation. eIF3 (and possibly other initiation factors) influences translation of *CLN3* because it is involved in all the steps along the formation of a preinitiation complex. Similarly, because the rapamycin-sensitive signal transduction pathway controls translation initiation (Barbet et al. 1996), it has a pronounced effect in Cln3p synthesis. Connections between these inputs are likely, because nutrients are known to affect the rapamycin-sensitive signaling pathway (Di Cono and Arndt 1996) and rapamycin also inhibits the function and synthesis of translation factors in mammalian cells (Terada et al. 1994; Beretta et al. 1996; Brown and Schreiber 1996; Brunn et al. 1997; Redpath et al. 1996).

cAMP regulates Cln3 protein levels

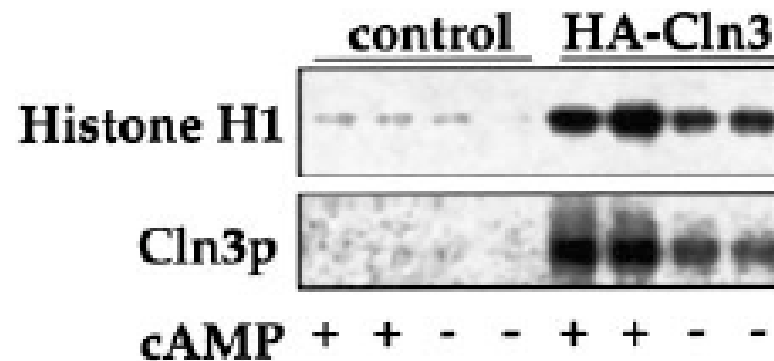


Fig. 4. cAMP regulates Cln3 protein and Cln3–Cdc28 activity levels. A *cyr1 Δ cln3 Δ* strain (TH122) carrying a plasmid expressing an epitope-tagged Cln3 protein from the normal *CLN3* promoter was grown in selective medium with 1 mM cAMP to mid-log phase. A sample was collected, the remaining cells were transferred to the same medium without cAMP and the cells were harvested after 1 h. Extracts were prepared from duplicate samples for immunoprecipitation of the tagged Cln3 protein with the monoclonal antibody 12CA5, and the immunoprecipitates were then used for kinase assays using histone H1 as a substrate (top), or Western blotting with the 12CA5 antibody (bottom). Control cells (TH5) carried an untagged *CLN3*.

Regulation of Cln3 protein level requires untranslated regions

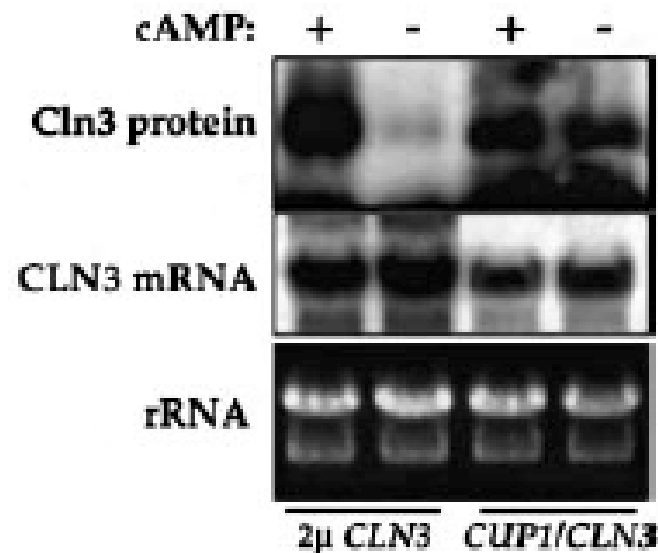
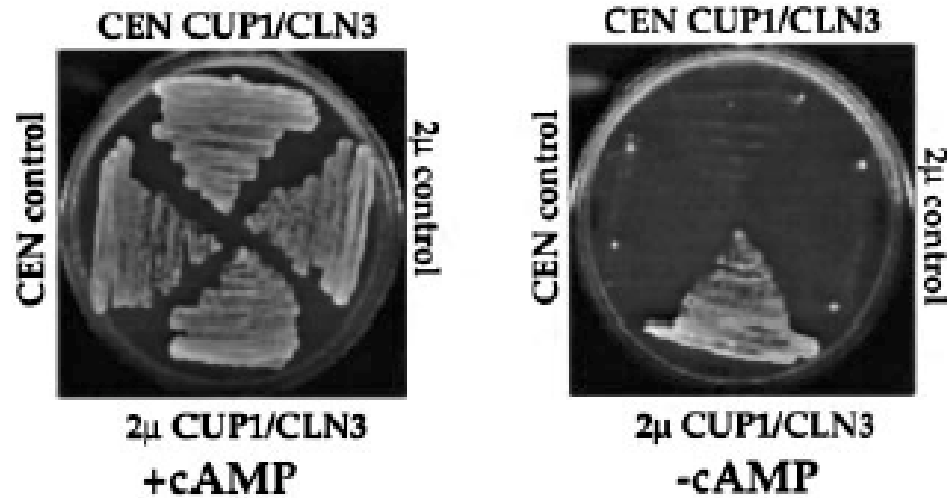


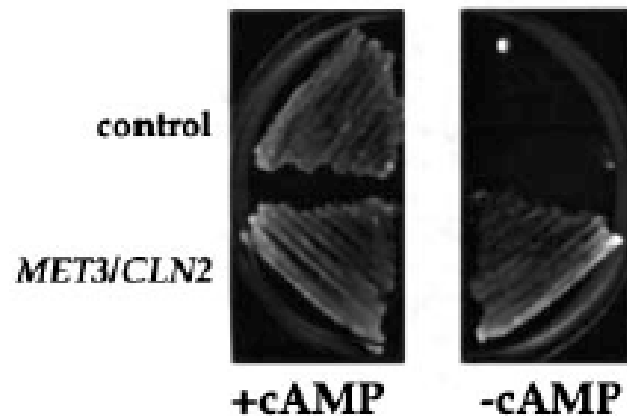
Fig. 5. Regulation of Cln3 by cAMP requires untranslated flanking regions. Strain TH122 carrying an epitope-tagged *CLN3* gene with normal flanking sequences on a 2μ plasmid (2μ CLN3) and TH100 carrying a 2μ plasmid with the *CUP1* promoter and *CYC1* terminator sequences in place of the normal flanking sequences (*CUP1-CLN3*) were grown overnight to mid-log phase in selective medium containing 1 mM cAMP. Cells were spun and resuspended to 0.4 OD₆₆₀ in fresh medium either with or without cAMP, as indicated, and grown for 1 h. Samples were collected for immunoprecipitation–Western blotting of the tagged Cln3, and for RNA preparation and Northern blotting. Numerical data from the densitometer scan of the Western blot and the phosphoimager scan from the Northern blot are shown in the lower panel.

Overexpression of Cln3 bypasses the requirement for cAMP

B

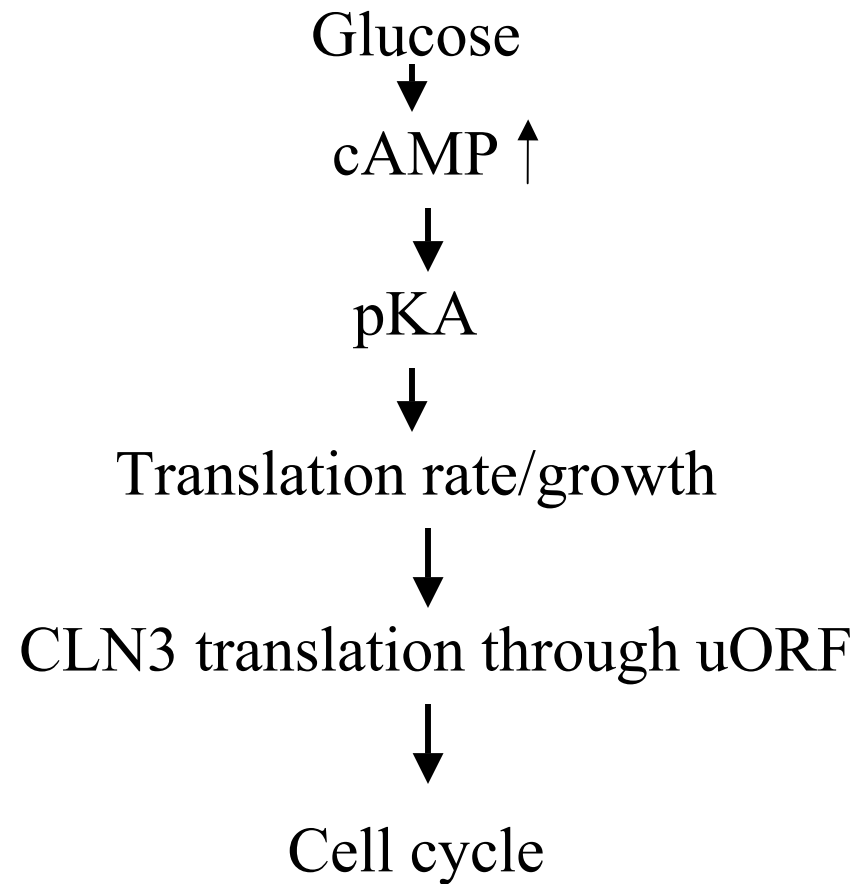


C

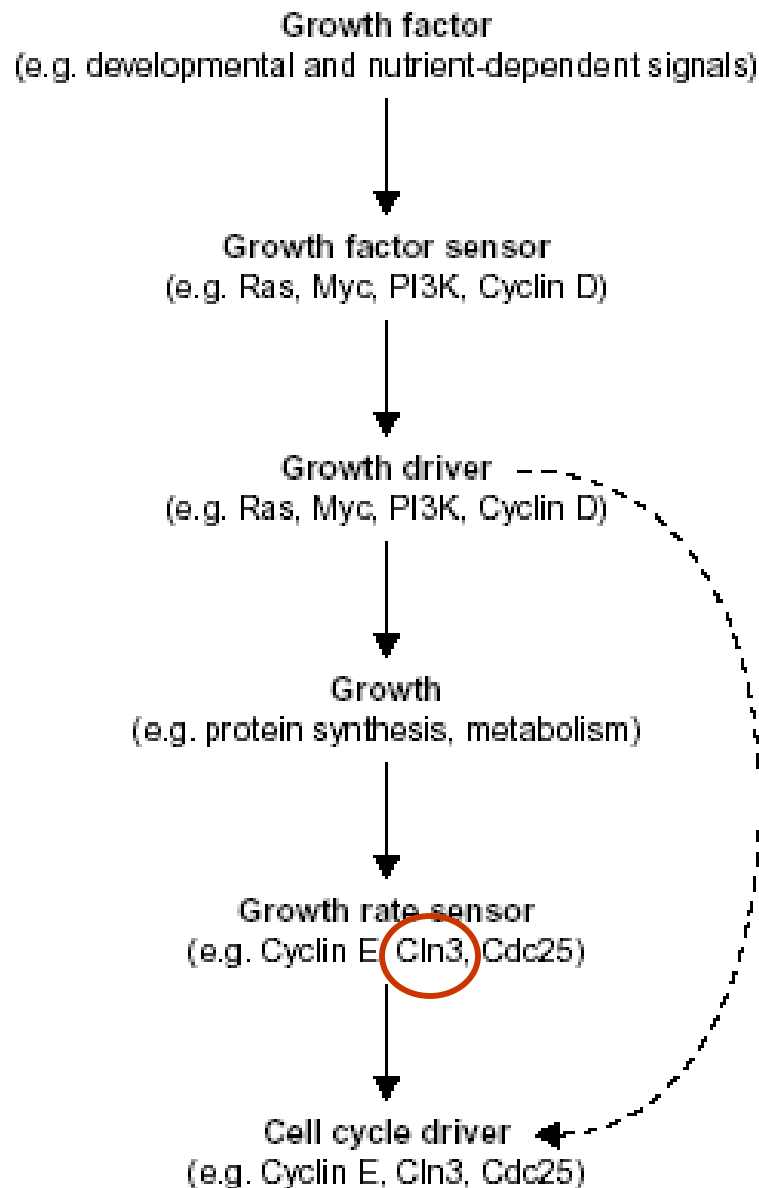


and *CUP1-CLN3* plasmid without cAMP (■). (B) The strains described above, along with strains carrying the same *CUP1-CLN3* construct in a CEN plasmid (pCU2) and the control CEN plasmid, were streaked onto either YEPD/1 mM cAMP or YEPD without cAMP. The plate with cAMP was incubated for 2 days at 30°C, the plate without cAMP was incubated for 3 days. (C) TH5 cells with either a plasmid carrying *MET3-CLN2* (pMET3-CLN2) (Espinoza *et al.*, 1994) or a control plasmid were streaked onto methionine dropout plates either with or without cAMP, as indicated, and incubated as in (B).

cAMP is a growth-regulating pathway in yeast



Growth is above the cell cycle



Glucose/ATP/GTP

pKA and TOR

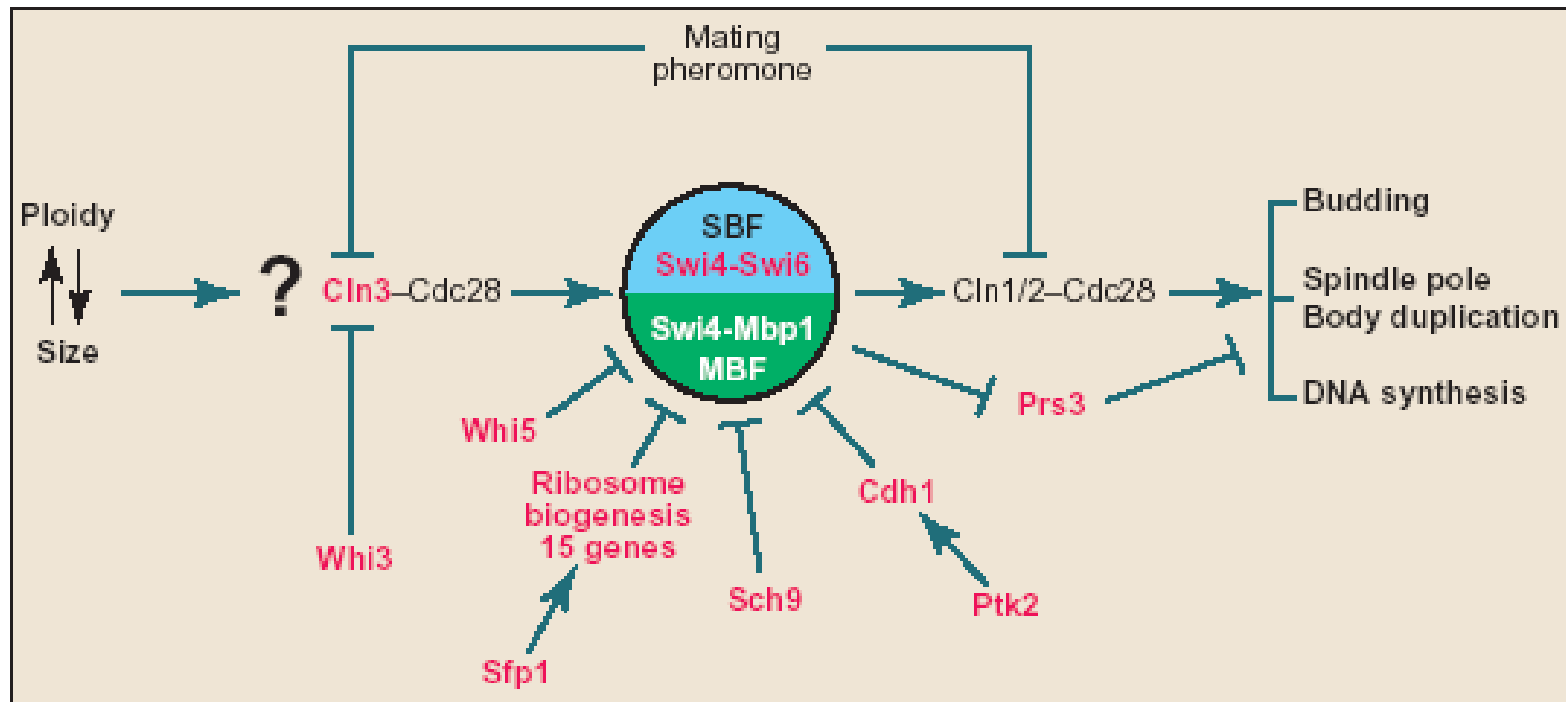
Hierarchy of growth control. Growth factors such as developmental and nutrient-dependent signals activate growth factor sensors such as Ras, Myc, PI3K and Cyclin D. These same proteins also drive growth by increasing rates of protein synthesis and by regulating other metabolic processes. Some growth drivers may also regulate the cell cycle via growth-independent mechanisms (dashed arrow). Growth-rate sensors, such as the G_1/S limiters Cyclin E and Cln3, and the G_2/M limiter Cdc25, sense cellular growth rates and drive cell-cycle progression. Combining the tasks of sensing growth factors and driving growth, or sensing growth rates and driving the cell cycle, ensures that these processes remain coupled.

Systematic identification of pathways that couple cell growth and cell division

5958 single gene deletion strains
(diploid)49 mutants (29 lge, 20 whi)
Gene dosage dependent (30%)

Unknown function (9)
Transcriptional regulation
Signal transduction
Cell cycle regulation
Protein translation

Systematic identification of pathways that couple cell growth and cell division



A great Start. The Start network in *S. cerevisiae*. Proteins identified in the screen of 6000 *S. cerevisiae* gene deletion mutants by Jorgensen *et al.* (5) are shown in red. The molecular mechanisms that enable yeast cells to measure their size against DNA content and then transmit this information to the Start network, which initiates mitotic cell division, are still not understood. Proteins implicated in this process include those involved in ribosome biogenesis.

As the rate of ribosome biogenesis is proportional to the cell size, it could be the measure that cells use to assess how big they are

Science 297. pp 351-352 (2002)

Cln3 activates the transcription of other cyclins

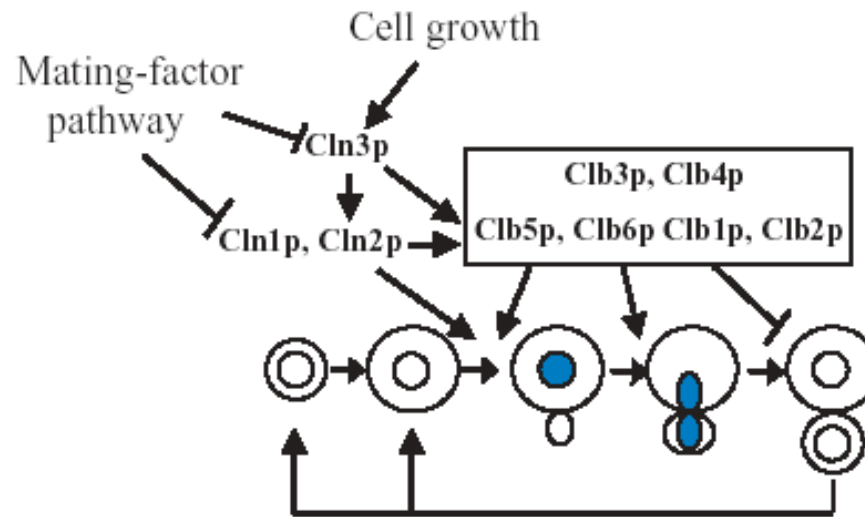


Fig. 1. Cyclins and control of the budding yeast cell cycle. All cyclins shown interact with the Cdc28p CDK. The Cln G1 cyclins are required for cell cycle Start, which entails bud emergence, inhibition of the mating factor pathway and activation of Clb-Cdc28p complexes. Cln3p is primarily a transcriptional activator of *CLN1*, *CLN2*, *CLB5* and *CLB6* and other genes (Breedon, 1996; Dirick et al., 1995; Levine et al., 1996; Stuart and Wittenberg, 1995; Tyers et al., 1993). The *CLN1 CLN2* gene pair may act directly to drive bud emergence and morphogenesis, as well as Clb-Cdc28p activation (reviewed in Cross, 1995). Cell cycle Start is modulated by cell growth and by the mating-factor pathway, probably through direct effects on Cln-Cdc28p function (Cross, 1995; Jeoung et al., 1998; Nasmyth, 1993; Nasmyth, 1996). Clb-Cdc28p complexes are directly responsible for activation of DNA replication and mitotic initiation (Nasmyth, 1996). Shaded circles represent nuclei undergoing DNA replication.

Microarray around the cell cycle in yeast

<u>3 synchronisation methods:</u>	800 genes cell cycle regulated
α -factor arrest	(out of around 6200)
Elutriation	G1: 300
Arrest of Cdc15 ts mutant	S: 72
	G2: 121
	M : 195
	M/G1: 113

Around half of these (400) responded to Cln3 (G1 cyclin or Clb2 (G2/M cyclin) overexpression.

400 000 individual measurements

Mol. Biol Cell 9, 3273-97 (1998)

Transcriptional regulatory networks in yeast

Chromatin immunoprecipitation experiment (ChIP) to identify promoters for transcription factors (106 out of 141)

- The promoter of 2343 of 6270 yeast genes were bound by at least 1 TF (on rich medium)
- 0-181 promoter/regulator (average 38)

RESEARCH ARTICLES

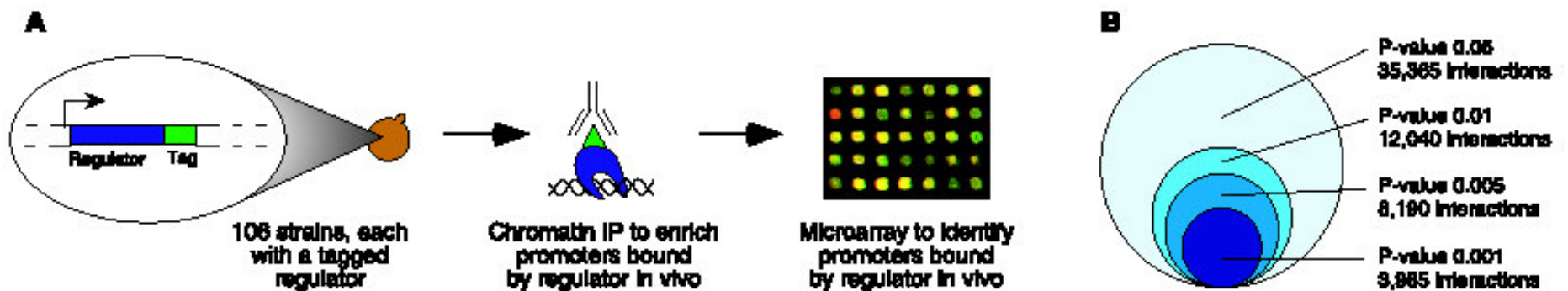


Fig. 1. Systematic genome-wide location analysis for yeast transcription regulators. (A) Methodology. Yeast transcriptional regulators were tagged by introducing the coding sequence for a *c-myc* epitope tag into the normal genomic locus for each regulator. Of the yeast strains constructed in this fashion, 106 contained a single epitope-tagged regulator whose expression could be detected in rich growth conditions. Chromatin immunoprecipitation (ChIP) was performed on each of these

106 strains. Promoter regions enriched through the ChIP procedure were identified by hybridization to microarrays containing a genome-wide set of yeast promoter regions. (B) Effect of *P* value threshold. The sum of all regulator-promoter region interactions is displayed as a function of varying *P* value thresholds applied to the entire location data set for the 106 regulators. More stringent *P* values reduce the number of interactions reported but decrease the likelihood of false-positive results.

Network motifs

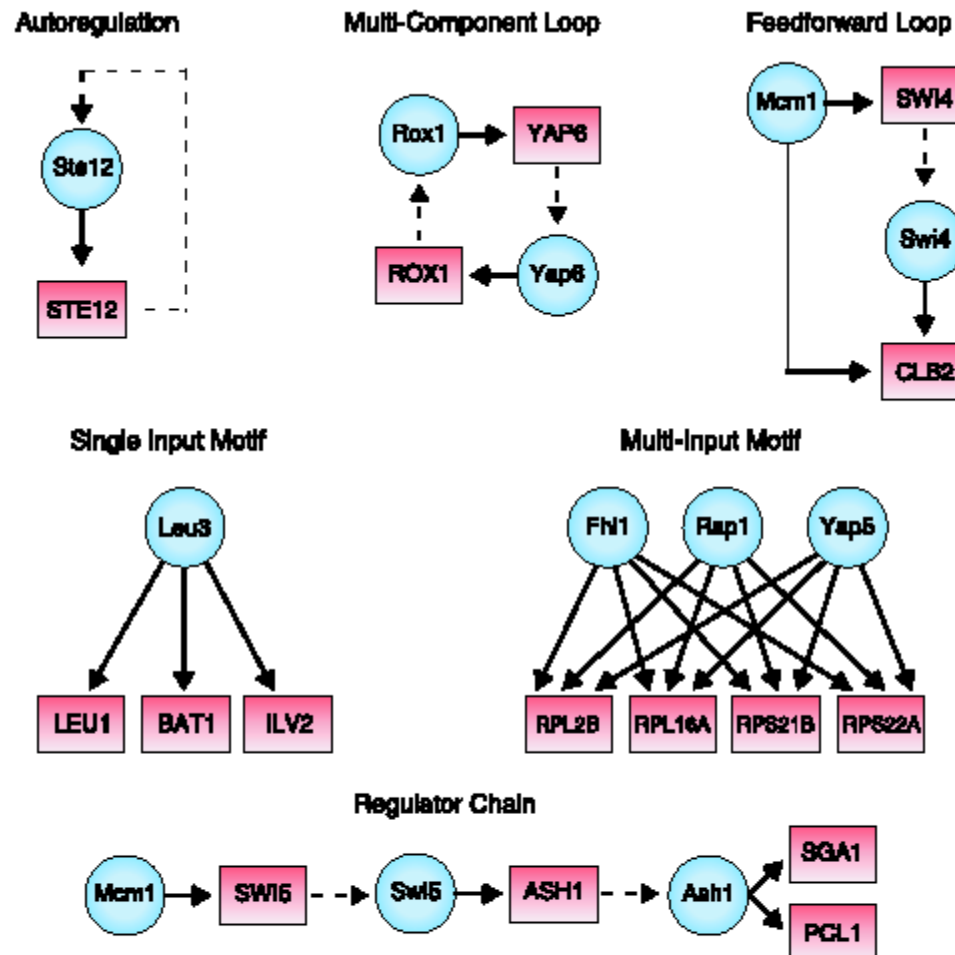


Fig. 3. Examples of network motifs in the yeast regulatory network. Regulators are represented by blue circles; gene promoters are represented by red rectangles. Binding of a regulator to a promoter is indicated by a solid arrow. Genes encoding regulators are linked to their respective regulators by dashed arrows. For example, in the autoregulation motif, the Ste12 protein binds to the *STE12* gene, which is transcribed and translated into Ste12 protein. These network motifs were uncovered by searching binding data with various algorithms. For details on the algorithms used and a full list of motifs found, see (18).

Network structure

Look for genes that are co-ordinately bound and expressed
(multi-input motives refined for common expression)

- Set of genes (G) bound by a set of regulators (S).
- Find a subset of genes in G which are similarly expressed over the entire set of expression data (core profile)
- The remainder of the genome is scanned for genes with similar expression profile to core
- Establish, if the set of regulators (S) are bound with combined and lower probability

Cell Cycle Network Structure

11 regulators were found

1. Correctly assigned all regulators to cell cycle phase
2. Found two new regulators and defined the function
3. Computational reconstruction of the regulatory architecture was automatic and required NO prior knowledge.

Network structure for the yeast cell cycle

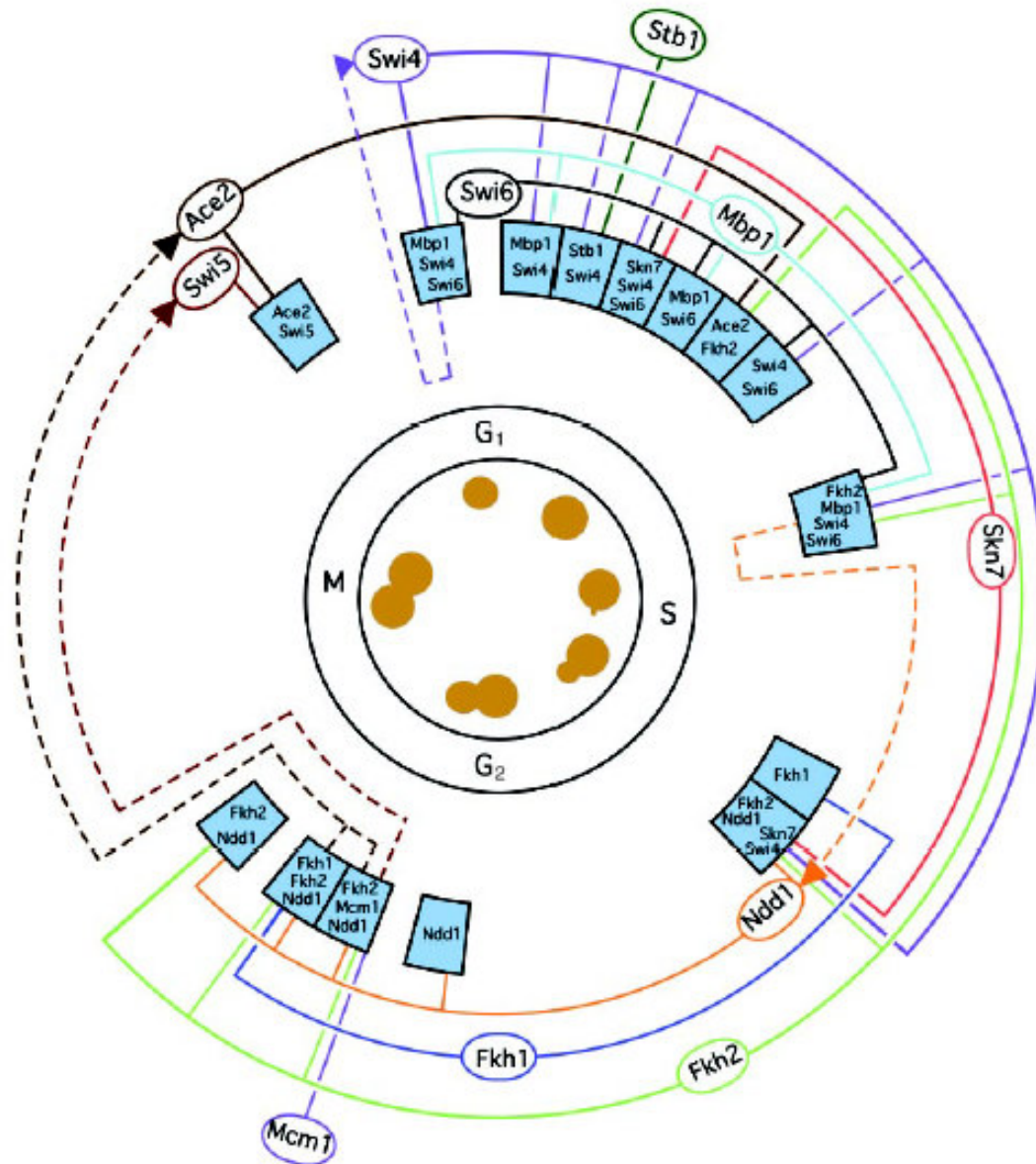


Fig. 4. Model for the yeast cell cycle transcriptional regulatory network. A transcriptional regulatory network for the yeast cell cycle was derived from a combination of binding and expression data (see text). Yeast cell morphologies are depicted during the various stages of the cell cycle. Each blue box represents a set of genes bound by a common set of regulators and co-expressed throughout the cell cycle. Text inside each blue box identifies the common set of regulators that bind to the set of genes represented by the box. Each box is positioned in the cell cycle according to the time of peak expression levels for the genes represented by the box. Regulators, represented by ovals, are connected to the sets of genes they regulate by solid lines. The arc associated with each regulator effectively defines the period of activity for the regulator. Dashed lines indicate that a gene in the box encodes a regulator found in the outer rings.

Preparation for the exam:

Lecture 1-3 (basic cell cycle, S-phase, M-phase)

Common regulatory principles for cell cycle processes
(phosphorylation, localisation, protein degradation).

Textbook (Lodish or Alberts).

Lecture 4 (Meiosis) How meiosis is different from mitosis,
and how it is initiated.

Lee and Amon (2001) Meiosis: how to create a specialised cell cycle.

Curr. Opin. Cell Biol. 13: 770-777

Lecture 5 (Pheromone signalling). Anatomy of a signalling pathway

Herskowitz. (1995) MAP Kinase pathways in Yeast:

For Mating and More. Cell 80. 187-197.

Lecture 6. (Growth). How growth is coupled to cell cycle

Polymenis and Schmidt (1999). Curr Opin. Gen. Dev. 9. 76-80.

Lecture 4-6. Transcriptional control, novel methods

Futcher (2002). Curr. Opin. Cell Biol. 14 676-683, and other references
during the lectures.