Control of the yeast cell cycle by the Cdc28 protein kinase

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It is becoming increasingly apparent that the diverse functions of Cdc28 during the yeast cell cycle are performed by forms of the kinase that are distinguished by their cyclin subunits. Entry into the cell cycle at START involves the Cln cyclins. S phase needs Clb5 or Clb6 B-type cyclins. Bipolar mitotic spindle formation involves Clb1–4 B-type cyclins. Much of the order and timing of the cell cycle events may involve the progressive activation of Cdc28 kinase activities associated with different cyclins, whose periodicity during the cycle is determined by both transcriptional and post-transcriptional controls.

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Introduction

The notion that a master regulator might orchestrate cell cycle progression stems primarily from cell fusion studies showing that the cytoplasm of M phase cells can cause nuclei from other stages to enter mitosis prematurely [1]. Biochemical and genetic searches for this regulator converged about 4 years ago with the discovery that an activity called maturation-promoting factor capable of causing frog extracts to enter M phase [2] was composed of a 34 kDa kinase. The homologues of this kinase, Cdc28 and cdc2, from two distant yeasts (*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, respectively) had previously been shown through genetic analysis [3,4] to have key roles in regulating cell division. It has since become clear that many, if not all, eukaryotic cells contain multiple forms of this kinase, some concerned with commitment to the cell cycle, others with S phase, and yet others with various aspects of mitosis. It is now thought that the sequential appearance of these different forms could determine the order of many key cell cycle events. This review discusses our current understanding of the functions and regulation of the Cdc28 kinase in the budding yeast *S. cerevisiae*, for it is here that the notion of multiple kinase forms was born.

The *Saccharomyces cerevisiae* cell cycle

S. cerevisiae cells divide by budding to produce progeny that differ in several respects. The mother cell is usually larger than the daughter cell derived from the bud because the time between emergence of the bud and its abcission from the mother cell is less than the mass-doubling time [5]. It has been suggested that the asymmetrical mode of division of *S. cerevisiae* is atypical and this could cause its cell cycle controls to be exceptional [6]. In fact, many if not most, eukaryotic cells produce non-identical progeny. This property is just particularly obvious in budding yeast.

The fate of newly born G_1 cells depends on their environment, their life cycle stage and their history. In the absence of nutrients, cells remain in G_1 and enter a quiescent state, during which protein synthesis is much reduced, cells have stores of glycogen, and acquire tolerance to heat shock [7]. The failure to enter a new cell cycle and the acquisition of heat tolerance are probably independent responses to the lack of nutrients because slow growing G_2 cells acquire similar levels of thermotolerance as slowly growing or stationary phase G_1 cells; that is, arrest in G_1 is not necessary for entry into a stress-resistant state (B Futcher, personal communication). In the presence of nutrients, G_1 cells enter S phase only after a period of growth, whose duration depends on cell size at birth and is longer in daughter than in mother cells [5].

During the early G_1 growth phase, haploid cells remain capable of conjugation, which is initiated by pheromones secreted by cells of opposite mating type. The mating pheromones not only induce genes involved in conjugation but also prevent cells entering S phase, with the result that mating partners arrest each other in G_1 [8]. Shortly before S phase, haploid cells become refractory to cell cycle arrest caused by pheromone and no longer require nutrients for completion of the cell cycle. This point, which is called START, divides G_1 into an early phase during which cells keep open several developmental fates, and a late phase in which cells are committed to completing the mitotic cell cycle [9]. The concept of

Abbreviations

SBF—Swi4/6-binding factor; **SCB**—Swi4/6 cell cycle boxes; **MBF**—MluI-binding factor; **MCB**—MluI cell cycle boxes.

START can also be applied to diploid cells, where it is defined as the point in G_1 before which cells can begin meiosis and after which cells are committed to a further mitotic division. Size homeostasis is maintained by the requirement that cells achieve a minimum size before they can undergo START.

Preparations for all aspects of the cell division process occur soon after cells undergo START (Fig. 1): cells enter S phase, they duplicate their spindle pole bodies, the first step to forming a mitotic spindle [10], and lay down proteins required for cytokinesis in the vicinity of the future bud site [11]. The behaviour of mutants shows that these three events are independent of each other. The *cdc4*, *cdc7*, *cdc34* and *cdc53* mutants cannot enter S phase but they bud and duplicate their spindle pole bodies normally [9,12], whereas *cdc31* and *cdc24* mutants are specifically defective in spindle pole duplication [13] and localization of proteins to the prospective bud site [14], respectively.

Defining entry into G_2 and M phases in *S. cerevisiae* has been a matter of some debate. It has been argued that S, G_2 and M phases overlap [6]. The early duplication of spindle pole bodies may be widespread in fungi [15] and is analogous to the duplication of the centrioles during S phase. It is therefore not a suitable criterion for determining whether a cell has entered M phase. Duplicated spindle pole bodies remain closely attached to each other for a large fraction (>25%) of the cell cycle and their separation to opposite poles of the nucleus and the formation bi-polar spindles does not occur until well after the completion of S phase, though significantly before nuclear division [10]. Thus, discrete S, G_2 and M phases clearly exist. What is less clear is whether the appearance of a nuclear spindle apparatus should be considered the beginning of M phase. Until recently, there has been no other criterion because the nuclear membrane does not break down (as in all fungi) and chromosome condensation could not be detected. However, recent observations using *in situ* hybridization (FISH) suggest that ribosomal DNA is condensed approximately threefold in cells treated with nocodazole compared to mutants that fail to complete S phase but nevertheless form bipolar spindles (V Guacci, E Hogan, D Koshland, personal communication). M phase might therefore be best considered to begin only when cells have formed bipolar spindles and condensed their chromosomes. Cells that have formed bipolar spindles but have not yet condensed their chromosomes could be considered to be in a prophase-like state. In contrast, anaphase is relatively easy to detect. After certain gyrations in the neck between mother cell and bud [16], the DNA mass can be seen splitting into two

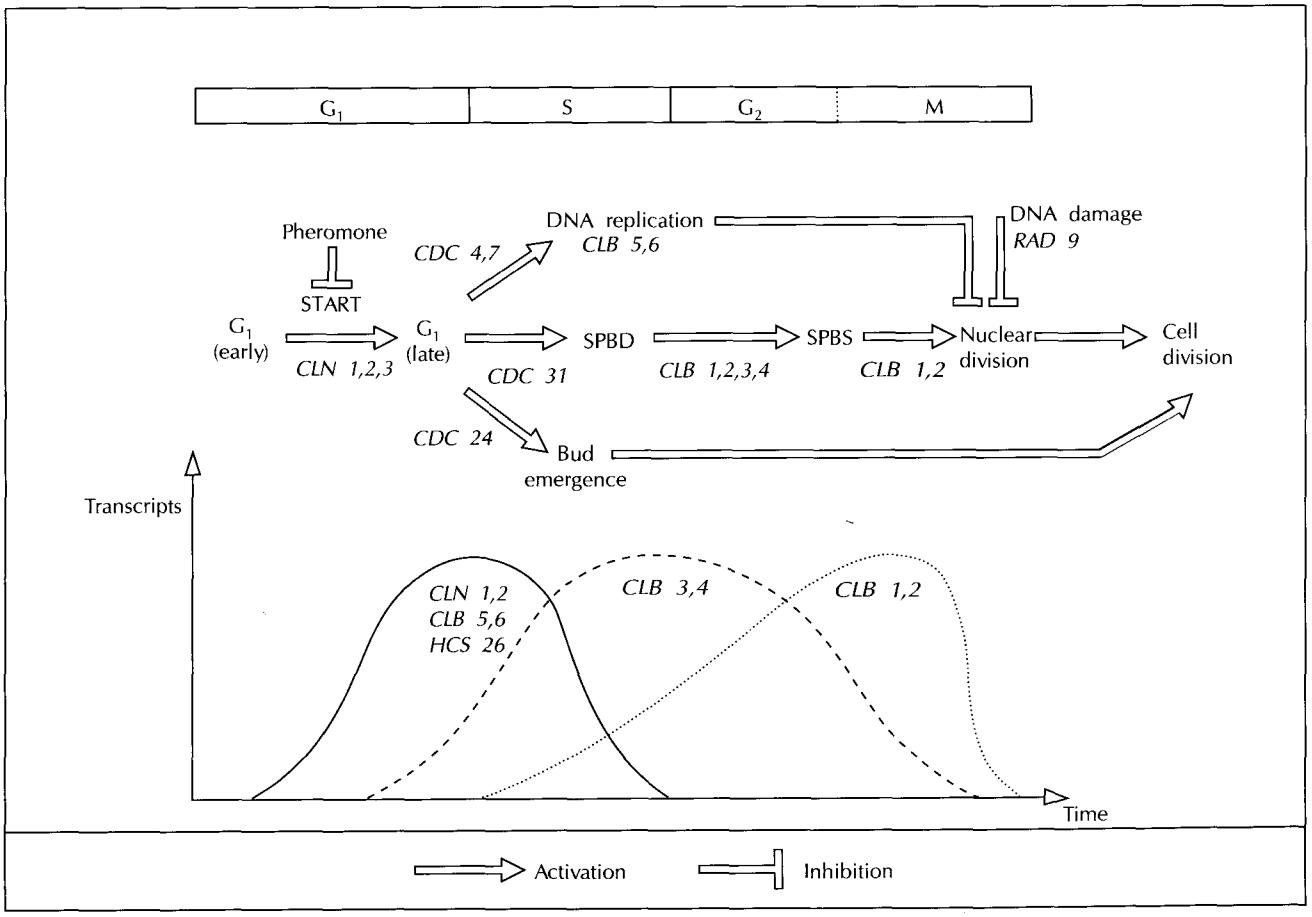

Fig. 1. The abundance of cyclin transcripts and their functions during the cell cycle of *S. cerevisiae*. SPBD, spindle pole body duplication; SPBS, spindle pole body separation (i.e. the formation of bipolar spindles).

as the spindle pole bodies move further apart and the nucleus becomes extended, a movement that would correspond to anaphase B. Whether anaphase A precedes this event is still unclear but this issue should be resolvable using FISH.

It is difficult to establish the phenotype of mutant cells without knowing their precise history. Cell cycle studies are therefore best performed using synchronous cultures. Three methods are particularly good for inducing synchrony: release from G_1 arrest induced by pheromone [17]; release from a late anaphase block imposed by a temperature-sensitive *cdc15* mutation [18]; and the manipulation of G_1 cyclin levels by their expression from the *GAL1–10* promoter [19••] All these methods are, however, prone to artefacts and results are best confirmed using cultures obtained by centrifugal elutriation. Pure populations of G_1 daughter cells can be obtained by elutriation as long as the cells are grown using suboptimal carbon sources (such as raffinose). Such cultures are particularly useful for comparing early and late G_1 events, but only a limited number of cells can be obtained.

The Cdc28 protein kinase

Fortune favoured yeast geneticists in their discovery of Cdc28. Of the forty or so cell cycle genes identified on the basis of mutant cells arresting uniformly at particular cell cycle stages, *cdc28* mutants were considered exceptional because they were the only ones defective in START despite normal rates of protein and RNA synthesis [20]. It is now recognized that *CDC28* has multiple roles during the cell cycle [21,22,23••], and there is still no good explanation why most alleles arrest uniformly in G_1; mutants that arrested throughout the cell cycle would have been ignored! Furthermore, other genes required for START have since been discovered (e.g. *CDC37* and *SIT4*). If they had been found at the same time as *CDC28*, there would have been little reason to concentrate on the latter. Better evidence for an important regulatory role had meanwhile emerged for the *cdc2* gene from the distantly related fission yeast *S. pombe*, in which there were alleles that advanced the timing of mitosis [24]. The discovery that *cdc2* and *CDC28* can complement each other and that they encode homologous protein kinases was therefore of fundamental importance in bringing together two different schools of cell cycle genetics [25–27].

Cyclins

Insight into how the Cdc2/Cdc28 protein kinase could be involved in such diverse functions as DNA replication and mitosis had to await the discovery that its animal homologue was an essential component of the previously known growth-related histone H1 kinase and of an activity (known as maturation-promoting factor) from *Xenopus* eggs capable of inducing *in vitro* metaphase arrest (reviewed in [28]). Cdc2, Cdc28 and their animal counterparts are only active as kinases when complexed with a class of proteins called cyclins, which were initially discovered by virtue of oscillations in their abundance during sea urchin cleavage divisions [29]. Studies with *Xenopus* extracts suggested that entry into metaphase was triggered by the appearance of kinase activity associated with B-type cyclins and that its destruction by proteolysis of the unstable cyclin subunit might initiate anaphase [30].

A very different type of cyclin, now called Cln3, was soon afterwards discovered in *S. cerevisae*. Mutations that stabilize Cln3 allow cells to undergo START at an abnormally small size [31] or in the presence of pheromone [32]. This led to the hypothesis that the G_1 and G_2 functions of Cdc2/Cdc28 are executed by forms of the kinase that differ according to the type of cyclin subunit associated with the kinase subunit; Cln and B-type cyclin associated kinases were postulated to be active at START and mitosis, respectively. At the time, this hypothesis suffered from two major flaws: first, unlike mutations in *CDC28*, deletion of *CLN3* merely delays START; second, no B-type cyclins had been detected in *S. cerevisiae*. Indeed, it was argued that mitosis in *S. cerevisiae* was abnormal in that it was initiated at START and might therefore use only abnormal (Cln) cyclins.

Both problems have since been solved by the discovery of many more cyclin genes in *S. cerevisiae* (Fig. 2). One family, composed of *CLN1*, *CLN2* and *CLN3* is essential for START [33], another, composed of B-type cyclins encoded by *CLB1*, *CLB2*, *CLB3* and *CLB4*, is necessary for the formation and function of the mitotic apparatus [23••,34••,35••]. *CLB5* and *CLB6*, which encode another pair of B-type cyclins, are important for S phase (E Schwob, personal communication) [36••] and two distant members of the family expressed in G_1, *HCS26* [37••] and *ORFD* (open reading frame D) [38], have no known function. The rest of this review will consider whether these cyclins are indeed activators of the Cdc28 kinase, whether the kinase activity associated with them is cell cycle dependent and if so how it is regulated, and finally what are the consequences for a yeast cell of changes in particular forms of Cdc28 kinase activity.

G_1 cyclins and START

CLN1 and *CLN2* were isolated due to their rescue of a *cdc28* mutant when overexpressed [39]. They encode closely related proteins (58% identity) that are only distantly related to Cln3 (23% identity). All three Cln proteins are very different from the B-type cyclin family involved in the control of mitosis (only 16–21% identity). None of the three *CLN* genes are essential for cell division. Furthermore, all double mutants are viable, although their cell size is greatly increased. However, deletion of all three genes is lethal and causes cells to arrest as unbudded G_1 cells [33] capable of conjugation [40]. At least one of the three *CLN* genes is therefore necessary for START.

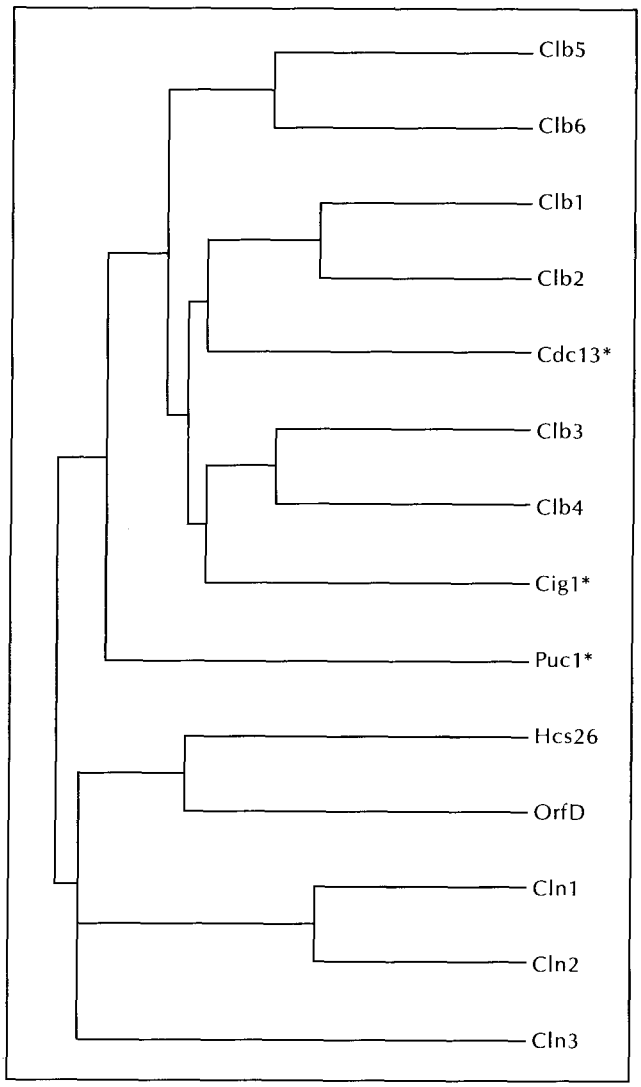

Fig. 2. A family tree of (complete) cyclin protein sequences from *S. cerevisiae* and *S. pombe*. The tree was constructed using the Wisconsin PILEUP program. *S. *pombe* cyclins.

Immunoprecipitates of each Cln protein contain a *CDC28*-dependent histone H1 kinase activity, which is much stronger for Cln1 and Cln2 than for Cln3 [41,42••,43]; this difference may be significant given their very different protein sequences. These data do not yet prove that the essential function of Cln proteins is to activate Cdc28, although they are certainly consistent with the notion. The Cdc28 kinase activity associated with Cln1 and Cln2 is tightly cell cycle regulated [43]; it is absent in cells arrested in G_1 by pheromone, but reappears soon after release, peaks in abundance as cells undergo START and declines as cells enter G_2. The abundance of Cln1 and Cln2 proteins and transcripts has a similar pattern [41], which might therefore be determined by transcriptional control. In contrast, there is little or no cell cycle variation in the level of Cln3 transcripts, protein, or associated Cdc28 kinase activity [43]. Expression of *CLN2* from the *GAL1–10* promoter reduces the length of G_1 (E Schwob, personal communication), suggesting that the onset of *CLN1* and *CLN2* transcription (as seen in wild type cells) may trigger START.

Regulators of *CLN1* and *CLN2* transcription

Two transcription factors called Swi4/6-binding factor (SBF) and MluI-binding factor (MBF; DSC1) have been implicated in late G_1-specific transcription (reviewed in [44]). They have a common regulatory subunit encoded by *SWI6* [45,46••–48••] but contain different site-specific DNA-binding subunits: Swi4 for SBF [49,50••] and a 120 kDa protein whose gene has not yet been characterized for MBF [46••]. The known binding sites for SBF and MBF, swi4/6 cell cycle boxes (SCBs) and MluI cell cycle boxes (MCBs), respectively, look different, but both proteins can bind at least weakly to each other's site [46••]. Both types of binding site are sufficient to confer late G_1-specific transcription on reporter genes and in some cases have been shown to be necessary for the transcription of genes whose promoters contain them [51,52•]. Current evidence suggests that SBF, at least, has an important role in activating *CLN1* and *CLN2*. Transcripts from both genes are nearly 10-fold less abundant in *swi4* mutants [37••,53••,54], which prevents either gene from fulfilling its function. A role for MBF cannot yet be excluded because there are no mutants defective only for this factor, *swi6* mutants being defective for both [46••,47••,55]. In certain genetic backgrounds, the lack of SBF function causes a lethality that is rescuable by *CLN2* expression from a foreign promoter [53••] or by increased *CLN2* gene dosage [37••]. Mutants lacking SBF can also be rescued by increased gene dosage of the *HCS26* gene [37••], which encodes yet another type of cyclin (with approximately 25% identity to Cln and B-type cyclins). Like *CLN1* and *CLN2*, *HCS26* expression occurs as cells undergo START (A Amon, personal communication) and depends on SBF, but it is not yet known whether it is an activator of Cdc28 or whether it has a role at START. *ORFD* [38] encodes a similar type of cyclin (T Hunt, personal communication) that may be similarly regulated [43].

A positive feedback loop

CLN1, *CLN2* and *HCS26* transcripts are absent throughout the early G_1 growth phase of daughter cells, but appear suddenly as cells undergo START (E Schwob, personal communication) [18,56••]. Insight into the abruptness of their activation came from the discovery that it depends on an active Cdc28 kinase [19••,56••], suggesting that a positive feedback loop may be involved. It has been proposed that Cln/Cdc28 kinase activates SBF, which in turn activates *CLN1/2* transcription, thereby closing the loop by further activating Cdc28 [37••,53••]. As predicted by this hypothesis, *CLN1* and *CLN2* activation is stimulated by G_1 cyclin activity [19••,56••]. However, the importance of the positive feedback loop in the activa-

tion of *CLN1* and *CLN2* in normal cells has not yet been established.

It is still not known how SBF is activated by Cdc28. A potential mechanism is the activation of *SWI4* transcription, which is also maximal in late G_1 [57••]. However, SBF appears to be quite stable [48••] and transcriptional activation of *SWI4* is unlikely to cause more than a twofold increase in its concentration. Furthermore, genes regulated by SBF can be at least partly activated by Cdc28 in the absence of protein synthesis [58••]. It is therefore likely that Cdc28 causes a post-translational modification of SBF. There is a change in the electrophoretic mobility of SBF–DNA complexes as cells undergo START, but it is not known whether this is due to the arrival a new protein (e.g. a cyclin) or due to phosphorylation of Swi4 or Swi6 by Cdc28 [48••].

A positive feedback loop may help explain the apparent irreversibility of START. It is to be expected that the SBF/G_1 cyclin regulatory circuit would have only two stable states: one with low kinase and a second with high kinase. The transition from the low to the high kinase state may be the biochemical basis to START. Repression of *CLN1* and *CLN2* as cells enter G_2 depends on the mitotic cyclins Clb1–4 (A Amon, personal communication).

Other genes required for START

Mutations in many genes cause yeast cells to arrest in G_1. In most cases, however, arrest can be explained as a secondary effect due to defective protein synthesis. For example, protein synthesis is greatly reduced in *cdc25* and *cdc35* mutants, which are defective in cAMP production (reviewed in ref [7]), whereas transcription from many *POLII* genes is defective in *cdc68* mutants [59]. There are in fact surprisingly few mutants whose G_1 arrest resembles that of *cdc28* or triple *cln* mutants, which continue to grow rapidly in the absence of cell division. Of these, the best characterized are *cdc37* [60] and *sit4* mutants [61]. The function of *CDC37* is unknown and *SIT4* encodes a phosphatase. Loss of *SIT4* function is only lethal in cells mutated in a second gene called *SSD1* (or *SRK1*), which is homologous to the *dis3* gene in *S. pombe* and could encode a phosphatase regulatory subunit. The *sit4 ssd1* double mutants normally arrest as unbudded cells in G_1, but expression of *CLN2* from a foreign promoter allows them to undergo S phase, although they still cannot bud [62••]. This suggests that Sit4 may have a role not only in activating G_1 cyclins (possibly via Swi4) but also in bud formation. It is interesting that both phosphatases and kinases are needed for START. Might the Sit4 phosphatase be required to prepare substrates for activation by the Cdc28 kinase?

Size control

START only occurs when cells reach a critical cell size [9]. Such a property ensures the coordination between cell division and growth, but how it is achieved is not yet understood. A good guess is that there are mechanisms for cyclin activation that are not dependent on the pre-existence of kinase activity and are somehow proportional to cell size. The Cln3 protein, which does not oscillate much during the cell cycle, is ideally suited for this function [43]. Deletion of *CLN3* is not lethal but causes cells to delay START until they are twice their normal size [31,32]. There may therefore exist more than one mechanism for triggering START. A Cdc28-independent pathway for activating *CLN2* transcription is another possibility [63].

It is worth considering how size control might function. Reducing protein synthesis with cyclohexamide delays START, suggesting that the critical parameter for initiation is not size *per se* but achieving a critical rate of protein synthesis (reviewed in [64]). G_1 cyclins are unstable proteins and their amount per cell will therefore reflect their rate of synthesis, which, like most other proteins, will be proportional to cell size. Thus, if Cln3 or proteins like it are accumulated in the nucleus, then their concentration (or amount relative to chromatin) will increase as cells grow. The dynamics of a positive feedback loop are such that it will be triggered from the low to high kinase state as soon as a critical level of an activator like Cln3 is reached, i.e. very small changes in the rate of Cln3 synthesis could trigger START. As predicted by this model, increased Cln3 expression from the *GAL1–10* promoter can trigger START prematurely in small daughter cells [43].

Nutrient control

There are two aspects to the control of START by nutrients. The first concerns the failure of cells to undergo START when starved [9]. So far, there is no evidence that G_1 cyclins or Cdc28 are directly affected by nutrient status, though it is entirely possible. However, there may be no need to invoke a special mechanism. G_1 arrest caused by starvation could be a simple consequence of lowered rates of protein synthesis (as discussed in [9]). The second aspect is of more obvious interest and concerns the control of cell size by nutrients. In general, cells growing in media that support fast rates of growth are larger than cells growing in poor media, an effect that is due to differences in the minimum size required for START [65]. It seems that fast-growing cells find it harder to undergo START than slow-growing cells, which is all the more striking when it is considered that the latter have lower rates of protein synthesis. A similar phenomenon has been reproduced in certain yeast strains by adding cAMP to the medium [66••]. In this case, the cell size required for START is increased by elevating cAMP levels,

i.e. cAMP acts as an inhibitor of START (which contrasts with the earlier notion that cAMP is an activator). In this context, it is striking that the levels of *CLN1* and *CLN2* transcripts are high in *cdc25* mutant cells arrested in G_1 [62••]. These cells have low rates of protein synthesis and presumably do not undergo START because G_1 cyclins are not synthesized and it is frankly surprising, in the light of the positive feedback model, that they transcribe *CLN1* and *CLN2*. The phenomenon could be explained if down regulation of *CLN1* and *CLN2* transcription by Cdc25 (via cAMP) was part of the mechanism by which nutrients controlled the cell size needed for START. The small size of *cdc25-1* mutants growing with a wild type generation time at the permissive temperature is consistent with this notion [67].

Is an oscillation needed for START?

The notion that a wave of Cdc28 activity might be responsible for START has been entertained ever since *CDC28* was found to encode a protein kinase. However, despite progress in understanding the dynamics of *CLN1* and *CLN2* regulation, it is still not clear whether an oscillation is essential for START. In *cln1 cln2* double mutants, *CLN3* is now essential, but this cyclin does not appear to be regulated. Furthermore, cells lacking all three G_1 cyclin genes can be kept alive by expression of *CLN1* or *CLN2* from constitutive promoters [33,53••]. The quality of life for such cells may be poor, but their survival in the laboratory suggests that a steady increase in cyclin activity could also accomplish START. This issue will not be resolved until we know more about post-translational controls on cyclin activity and more about other genes contributing to START whose activity is regulated. For example, evidence that Cln3 is activated by phosphorylation raises the possibility of a positive feedback loop involving auto-phosphorylation [42••]. Furthermore, cells lacking the regulated *CLN1* and *CLN2* genes still contain *HCS26*, *CLB5* and *CLB6*, which encode tightly cell cycle regulated cyclins possibly active at START (E Schwob, personal communication).

Regulation of START by pheromone

The activation of genes involved in conjugation (such as *FUS1*) and the inhibition of START are, by and large, independent responses of haploid cells to their mating pheromones. *FUS1* induction is not dependent on cell cycle arrest and there exist mutants that induce *FUS1* normally but nevertheless fail to undergo arrest (see below). Both responses rely on the same signal transduction pathway (reviewed in [8]). Pheromone binding to surface receptors releases the βγ-subunit of a G protein from an inhibitory α subunit, and this somehow activates a kinase cascade, through which the Fus3 kinase (which is homologous to mammalian MAP kinases) is activated by the Ste7 kinase [68].

Dominant alleles of *CLN3* that produce a more stable protein [32,42••] or recessive mutations that inactivate the *FAR1* gene [69] allow cells to continue division despite inducing genes like *FUS1* normally. The pheromone resistance of *far1* mutants depends on a functional *CLN2* gene. Pheromone-induced G_1 arrest is therefore thought to involve the inhibition of G_1 cyclin activity and the inhibition of *CLN2* at least depends on *FAR1*. *CLN1* and *CLN2* transcripts decline when cells are treated with pheromone [41], but low-level expression of *CLN2* from an unregulated promoter does not cause pheromone resistance (G Ammerer, personal communication) [70]. Thus, the inhibition of *CLN2* activity (by *FAR1*) must occur at a post-transcriptional level. Indeed, inhibition of Cdc28 may be due to a pheromone-induced association of the Far1 protein with Cln1 and Cln2 (M Tyers, B Futcher, and M Peter, A Gartner, J Horecker, G Ammerer, I Herskowitz, personal communications). Repression of *CLN1* and *CLN2* transcription could be a secondary consequence of inhibiting G_1 cyclin activity, as predicted by the positive feedback loop model. It could nevertheless contribute to the efficiency of arrest because high level expression of *CLN2* does cause pheromone resistance. *FAR1* transcripts are induced by pheromone, but this is not the sole mechanism by which Far1 activity is activated, because *FAR1* expression from the *GAL* promoter does not cause G_1 arrest. It is possible that Far1 is activated due to phosphorylation by the Fus3 kinase [68]. How Cln3 stabilization causes pheromone resistance is still unclear because overproducing the wild type protein from the *GAL* promoter does not generate the same phenotype [42••].

Consequences of G_1 cyclin activity

Little is known about physiological substrates for Cln/Cdc28 kinases. S phase entry occurs soon after the appearance of *CLN1/2* transcripts and requires the *CDC4*, *CDC7*, *CDC34* and *CDC53* genes, none of which are required for START in haploids [9] or for the activation of G_1 cyclins. *CDC34* encodes a ubiquitin conjugating enzyme, which has led to the proposal that degradation of G_1 cyclins might be necessary for the transition from START into S phase, just as the degradation of G_2 cyclins is necessary for exit from mitosis [71]. Cells arrested by *cdc34* do contain high levels of *CLN1* and *CLN2* transcripts (A Amon, personal communication) and have greatly elevated Cdc28-dependent kinase activity associated with Cln3, which may be due to the accumulation of a hyper-phosphorylated form [42••]. However, several facts seem at present inconsistent with the notion that DNA replication is hindered by high G_1 cyclin activity: high level constitutive *CLN2* expression functions to accelerate rather than delay S phase (E Schwob, personal communication); *cdc34* arrest does not depend on *CLN3* [42••]; and S phase occurs normally in other mutants in which Cln1 and Cln2/Cdc28 kinases fail to be repressed (A Amon, personal communication). *CDC4* encodes a protein homologous to β-transducins [72]

and *CDC7* encodes a protein kinase [73], but little is known about their function or whether they might be substrates for Cdc28. A complex that binds origins has recently been isolated [74••]. One or more of its constituents could be a substrate for Cdc28.

Many genes involved in DNA replication are activated transiently as cells undergo START (reviewed in [75]). Their transcription does not require *SWI4* but is de-regulated in *swi6* mutants and depends on MCBs, implicating MBF (and not SBF) in their regulation [46••,47••]. Gene activation by MBF requires the Cln G_1 cyclins and Cdc28. Most of the genes regulated by MBF encode stable proteins whose inheritance from the parental cell is sufficient for S phase. Nevertheless, MBF probably also regulates *CLB5* and *CLB6*, which encode B-type cyclins involved in S phase entry (E Schwob, personal communication). Thus, MBF activation may play an important part in the initiation of DNA replication.

It is not known how the activation of a Cln/Cdc28 kinase leads to the duplication of spindle pole bodies (Fig. 1) or to bud emergence. Neither of these events require the known G_2 cyclin genes *CLB1*, *CLB2*, *CLB3* and *CLB4* [34••], and they could therefore be under direct control of G_1 cyclins. Over-expression of stable G_2 cyclins not only causes mitotic arrest but also inhibits bud formation [76••], a phenomenon that could be explained if G_1 cyclins have a direct role in bud formation, which is hindered by an excess of G_2 cyclins.

G_2 cyclins and mitosis

S. cerevisiae contains at least four genes (*CLB1–4*) encoding B-type cyclins that are involved in mitosis. They comprise two families: *CLB1/CLB2*, which are 62% identical, and *CLB3/CLB4*, which are 50% identical (Fig. 2). The Clb1/2 pair is the more closely related to B-type cyclins from animals. Of the four genes, *CLB2* seems to have the most important role in mitosis. Deleting *CLB1*, *CLB3* and *CLB4* has little or no effect on cell division [34••,35••], whereas deletion of *CLB2* alone greatly delays the onset of mitosis [23••]. The phenotype of cells lacking activity of all four genes has been analyzed using strains whose *CLB1*, *CLB3* and *CLB4* genes are deleted and whose *CLB2* gene is conditionally active due either to a temperature-sensitive mutation (A Amon, personal communication) or to expression from the *GAL* promoter (which can be repressed) [34••]. Upon inactivation of *CLB2*, cells arrest in G_2 with duplicated spindle pole bodies and large buds. The duplicated spindle pole bodies remain attached, as they do in *cdc4* mutants, and consequently no bipolar spindle is formed. G_1 cells were seen to initiate and apparently complete S phase in the absence of *CLB1*, *CLB2*, *CLB3* and *CLB4*. In a separate study, in which a quadruple *clb* mutant was kept alive by expression of *CLB1* from the *GAL* promoter [35••], cells also seemed to have difficulties in completing S phase when *CLB1* was repressed.

Whereas *CLB3* and *CLB4* can be deleted with little or no effect on cell cycle progression, mutation of *CLB1* and *CLB2* is almost lethal and causes cells to arrest with bipolar spindles (A Amon, personal communication) [23••]. The *CLB1/2* and *CLB3/4* cyclin types therefore have different properties; either the *CLB1/2* pair or the *CLB3/4* pair can promote the separation of duplicated spindle pole bodies and the formation of a bipolar spindle, but the *CLB1/2* pair is necessary for subsequent stages of mitosis, which could include chromosome condensation or aspects of kinetochore function. At the moment, it would seem as if *CLB2* may be able to perform both early and late functions on its own. A caveat to this conclusion is that we do not know for sure whether *CLB1–4* are the only B-type cyclin genes active in mitosis in yeast. There may well be other genes, without whose function *CLB2* cannot alone drive cells through mitosis.

Cell cycle regulation of G_2 cyclins

Immunoprecipitates of Clb1, Clb2, Clb3 or Clb4 have *CDC28*-dependent histone H1 kinase activities that are tightly cell cycle regulated and seem much stronger than those found associated with G_1 cyclins [43,77••,78]. Clb2-associated kinase is absent in cells arrested in G_1 by pheromone. Its appearance upon release occurs after DNA replication (around the time that cells form a bipolar spindle) and precedes anaphase by at least 15 min [79]. Clb2 protein and kinase activity are destroyed as cells exit from mitosis, as is found for B-type cyclins in other eukaryotes. Clb1 kinase is similarly regulated, but Clb3 and Clb4 kinases appear somewhat earlier [78].

CLB1 and *CLB2* transcripts have a similar profile to Clb2 kinase activity, appearing in G_2 and disappearing as cells enter G_1 [23••,76••]. *CLB3* and *CLB4* transcripts appear earlier (but still later than *CLN1* and *CLN2*), which is consistent with their function in an earlier stage of mitosis [34••,35••]. How much transcriptional control contributes to the regulation of Clb/Cdc28 kinase activity is not yet known because the consequences of premature activation or delayed repression have not yet been carefully analyzed. Little is known about the transcription factors responsible for regulating *CLB* transcription. *CLB1* and *CLB2* regulation appears very similar to that of the *SWI5* gene, whose promoter requires the formation of a ternary complex containing Mcm1 and a factor called Swi five factor (SFF) [80]. Of these, SFF is the better candidate for a G_2-specific transcription factor, because Mcm1 is also involved in the activation of genes that are not cell cycle regulated [81].

An important question is whether the *RAD9*-dependent inhibition of mitosis by DNA damage [82] (Fig. 1) is due to regulation of one or another form of the Cdc28 kinase. DNA damage arrested cells, e.g. *cdc13* mutants, arrest with bipolar spindles, a phenotype that is similar to that of *clb1 clb2* double mutants. However, Cdc28 kinase activity associated with Clb2 in arrested *cdc13* mutants is at least 50% of that found in cells arrested with nocodazole [77••]. The possibility that mitosis can be regulated

by means that do not directly involve Cdc28 must therefore be seriously considered.

Roles of Cdc28 phosphorylation and cyclin proteolysis

In several other eukaryotes, Cdc2/Cdc28 kinase activity associated with B-type cyclins is largely determined by post-transcriptional controls (reviewed in [28]). In *S. pombe*, Cdc2 kinase is inhibited during G_2 by phosphorylation of Tyr15 by the Wee1 family of kinases. Dephosphorylation by the Cdc25 phosphatase seems to determine the onset of M phase. Mutation of Tyr15 or the *wee1* gene causes premature entry into M phase. The equivalent residue in Cdc28 (Tyr19) is also phosphorylated in a cell cycle dependent manner [77••,83••]. Moreover, there exist homologues to the Wee1 kinase (B Booher, personal communication) and the Cdc25 phosphatase (called *MIH1* [84]). Despite this, mutation of Tyr19 to Phe has little or no effect on the length of G_2 or the inhibition of nuclear division by unreplicated or damaged DNA [77••,83••]. It would seem that the stoichiometry of Cdc28 Tyr19 phosphorylation is not sufficient to affect Cdc28 kinase activity greatly, although it appears that the kinase can be inhibited and cells prevented from undergoing nuclear division if the stoichiometry of phosphorylation is increased by over-expressing *S. pombe* Wee1 kinase in *mih1* mutants [84].

Tyr19 is phosphorylated in cells prevented from undergoing nuclear division due to unreplicated or damaged DNA (e.g. *cdc13* mutants) but is unphosphorylated in cells arrested due to microtubule defects (e.g. in nocodazole) [77••]. FISH analysis suggests that ribosomal DNA is less condensed in arrested *cdc13* mutants than in nocodazole (V Guacci, E Hogan, D Koshland, personal communication). Thus both the analysis of Cdc28 phosphorylation and the state of ribosomal DNA condensation suggest that *cdc13* arrest is G_2-like and nocodazole arrest M phase-like. Because *cdc13* mutants have bipolar spindles, they may be better considered as arresting in prophase.

The abrupt destruction of B-type cyclins during anaphase in *Xenopus* is conferred by a conserved sequence towards their amino termini, called a destruction box [30,85]. All four mitotic Clb cyclins in yeast contain sequences similar to the canonical destruction box, and mutation of the sequence in *CLB2* causes stabilization [79]. It therefore seems likely that the destruction machinery is conserved between *Xenopus* and yeast. Cyclin variants that cannot be destroyed because of mutations in their destruction boxes are reported to cause *Xenopus* extracts to arrest in metaphase, and this has led to the proposal that destruction of B-type cyclins signals the metaphase to anaphase transition [30]. Expression of stable versions of Clb1 or Clb2 cause a lethal cell cycle arrest in yeast, but the arrest does not correspond to metaphase. Cells arrest with greatly extended bipolar spindles and DNA masses that seem to have completely segregated, indicating that anaphase B at least has occurred [79]. Another piece of evidence inconsistent with anaphase being triggered by cyclin destruction is the behaviour of *cdc15* mutants, which arrest in a cell cycle state similar to that caused by stable cyclins. There is little or no drop in Clb2/Cdc28 kinase activity as *cdc15* cells undergo anaphase [79]. The kinase only disappears when cells are allowed to exit from late anaphase/telophase upon return of the mutant cells to the permissive temperature. Thus, cyclin destruction in yeast (at least) seems required for the final exit from mitosis and not, as previously thought [76••], for the initiation of anaphase.

Targets

Very little is known about physiological targets of mitotic forms of Cdc28. The only example so far is the Swi5 transcription factor, which accumulates in the cytoplasm during G_2 and M phases and only enters the nucleus as cells enter G_1 [86]. The nuclear localization signal of Swi5 is phosphorylated by Cdc28 and this prevents nuclear uptake [87••]. Destruction of the kinase at the end of mitosis leads to dephosphorylation and entry into the nucleus. Swi5 is required for transcription of the *HO* endonuclease gene involved in mating type switching and does not have an essential cell cycle function [88]. However, a related transcription factor, called Ace2, may be regulated in a similar manner [89••] and is required for the activation of a chitinase gene (*CTS1*) involved in cell separation as cells exit from mitosis (R Siegmund, personal communication). The activation of transcription factors through destruction of the Cdc28 kinase at the end of mitosis may be quite a general phenomenon. Several genes, in addition to *CTS1*, are only transcribed as cells enter G_1 [18]. The regulation of these early G_1 genes is distinct from those activated in late G_1 as cells undergo START, in that Cdc28 seems to repress the former but activate the latter.

Conclusions and questions

The budding yeast *S. cerevisiae* has at least 11 different genes that encode cyclin-like proteins, many of which have been shown to be associated with an active Cdc28 kinase. Some genes are required for START, others for DNA replication, and others for various steps in the assembly and function of a mitotic spindle (Figs 1 and 2). If we compare the cell cycle to a clock, it would seem that changes in Cdc28 activity not only sound the passing of the cell cycle 'day' (i.e. the passing from one cell cycle to the next) but also register the hours within that day.

Much remains to be discovered about the extent to which the timing of cell cycle events is determined solely by the activation of different cyclins. Only in the case of G_1 cyclins is there evidence that premature cyclin activation can actually advance cell cycle events. Furthermore, it is still quite unclear what distinguishes the activity of differ-

ent types of cyclins. What property of G_1 cyclins enables them exclusively to activate transcription at the beginning of the cell cycle and what property enables G_2 cyclins to promote the formation of a bipolar spindle? To what extent is this due to different substrate specificities and to what extent is this due to them being active at the correct time and in the correct cellular compartment? Identifying important substrates for different forms of the kinase should be a high priority in future studies.

Major questions remain concerning the dynamics of cyclin oscillations. Our current picture is as follows. Cells that have just entered G_1 start off with possibly only Cln3 protein, whose amount increases as cells grow; at some critical cell size *CLN1* and *CLN2* transcription is partially activated. Cln1 and Cln2 proteins then reinforce Cln3, leading to more *CLN1/CLN2* transcription and thereby to high levels of Cln/Cdc28 kinase activity. At some point during this process, the Cln/Cdc28 kinase activates transcription of other cyclin genes (like *HCS26*, *CLB5* and *CLB6*) and a battery of genes involved in DNA replication. Somewhat later, *CLB3* and *CLB4* transcripts appear and, later still, those from *CLB1* and *CLB2*. Meanwhile, transcription of *CLN1*, *CLN2*, *HCS26*, *CLB5* and *CLB6* declines. *CLB1* and *CLB2* transcripts and kinase activity remain until some event during anaphase causes B-type cyclins with destruction boxes to be degraded and their genes to be repressed. Little is known about post-transcriptional controls over Cdc28 kinase activity associated with either Cln or Clb proteins, what causes the decline of *CLN* and the rise of *CLB* transcription as cells enter G_2, and what causes the destruction of B-type cyclins, which is so important for exit from mitosis and for re-setting the cell cycle clock.

Another question of fundamental importance is the extent to which the successive waves of cyclin oscillations are dependent on each other rather than on the completion of events set in motion by earlier cyclins. For example, what role do G_1 cyclins have in activating G_2 cyclins and what role do they in turn have in repressing G_1 cyclins? Must events such as spindle pole body duplication and DNA replication be complete before G_2 cyclins can be activated? It would seem not in the case of the latter, because Cdc28 kinase associated with Clb2 becomes elevated in *cdc7* mutants, which cannot initiate DNA replication. The only aspect of cyclin control that is clearly dependent on the completion of cell cycle events is the destruction of mitotic cyclins following nuclear division. Many mutants defective in DNA replication or mitosis do not proceed to destroy mitotic cyclins. Enzymes involved in DNA replication, for example, are unlikely to be directly involved in cyclin proteolysis, so the cell must somehow register the failure of DNA replication and communicate this (however indirectly) to the destruction machinery. Such mechanisms have been called checkpoint controls [90].

It is in fact surprising how many mutants totally defective in nuclear division proceed with the activation and destruction of mitotic cyclins as if nothing had gone wrong with mitosis (e.g. [91]). Indeed, it is now clear that the initial *cdc* mutants collected by Hartwell [3] were highly biased by the requirement (a vital one at the time) that cells arrest with a uniform morphology. Many, if not most, mutants with defects to do with cell division do not in fact arrest uniformly because the cells cannot register the defect and shut down the oscillations of cyclin/Cdc28 activity. It seems that yeast cells check only certain cell cycle events (which include DNA damage [82] and the integrity of microtubules [92••,93••]), presumably those that are most likely to go wrong in the natural world. In this regard, yeast may not be that different from early frog embryos whose cyclin/cdc2 oscillations do not even require a nucleus [94].

Comparisons

The notion that different cell cycle events are regulated by forms of the Cdc2/Cdc28 kinase distinguished by their cyclin subunits may prove to be a conserved feature of the eukaryotic cell cycle, although the details differ considerably. In vertebrate cells, there exist at least four classes of cyclins [95]: E-type cyclins, which appear in late G_1, may be analogous to Cln1 and Cln2; A-type cyclins, which appear at the beginning of S phase and seem important for its completion, may be analogous to Clb5; B-type cyclins, which appear in G_2 and disappear as cells undergo anaphase, are similar to Clb1 and Clb2; and D-type cyclins, which are less tightly cell cycle regulated, could in this regard be analogous to Cln3. Of these different classes, only the B-type cyclins are obviously homologous when their primary sequences are compared. For example, the sequences of G_1-specific cyclins in vertebrates and yeast are not more related to each other than either is to B-type cyclins from either organism. Another difference between yeast and vertebrates is that the latter not only have multiple cyclin types but also multiple Cdc2/Cdc28 kinase subunits. Early cyclins seem to associate with the Cdk2 kinase subunit [96,97], whereas late cyclins associate with Cdc2.

In *S. pombe* as in *S. cerevisiae*, a single Cdc2 kinase subunit is required for both START and mitosis [98]. Three cyclin-like genes have been reported (Fig. 2): a B-type cyclin encoded by *cdc13*, which is required for mitosis [99]; a second B-type cyclin called Cig1, which is most closely related to Clb3 and Clb4 [100••] and seems important for S phase; and Puc1 which is related to *S. cerevisiae* G_1 cyclins but whose function is unknown [101]. The role of cyclins in START has therefore not yet been established. One reason for believing that the mechanism of START may be similar to that found in *S. cerevisiae* is that the *cdc10* gene [4], which is required for START, is homologous to the *S. cerevisiae SWI6* gene [45] and is a component of an MBF/DSC1-like factor that binds to MCB elements [102••]. It seems that transcriptional activation plays an important part in both yeasts. What is still unclear is what genes are activated by the *S. pombe* MBF/DSC1.

Even though the cell cycle components may be similar between *S. pombe* and *S. cerevisiae*, the way that they are used to regulate the cell cycle is very different. The

life cycle of *S. pombe* is such that it has a haploid vegetative phase [6], which may explain why it spends as little time as possible in G_1, when haploid cells are particularly sensitive to DNA damage [63]. Both size and nutrient controls in *S. pombe* are therefore exerted in G_2 and rely on control of Cdc2 phosphorylation, which is relatively unimportant for *S. cerevisiae* [77••,83••].

Certain conclusions can be drawn from comparing cell cycle control in these organisms: first, G_1 cyclins are much less conserved than the B-type cyclins involved in mitosis; second, in fungi at least, B-type cyclins have very diverse functions; third, the machinery for cyclin destruction at the end of M phase may be highly conserved; and finally, the enzymology for controlling phosphorylation of Tyr15 in Cdc2/Cdc28 is also highly conserved, even though the uses to which it is put are not. The universal instability of cyclins presumably enables signals from outside or inside the cell to affect rapidly their concentration and thereby biological activity. One suspects that our distant ancestors used a B-type cyclin to beat out its cell cycle rhythm with periodic cyclin proteolysis being a fundamental aspect of the oscillator (i.e. the heart of the cell cycle). Cdc2/Cdc28 tyrosine phosphorylation may have served to distinguish S from M phase. It must be significant that the more ancient aspects of cell cycle control are used to regulate mitosis. Is this because the fundamental mechanisms that regulate DNA replication evolved before the evolution of eukaryotic organisms? If this is the case, cyclins and Cdc2/Cdc28 may have evolved originally to regulate mitosis and have only more recently been used to exert control over DNA replication and passage through G_1. An alternative explanation is that the common ancestor of eukaryotes was a haploid organism, in which control of the cell cycle was exerted predominantly in G_2.

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This paper addresses the cell cycle regulation of SBF activity. Two forms of SBF are detected, which are distinguished by their electrophoretic

mobility. One form is absent in early G_1 cells, appears at the time of *HO* activation, but persists until the end of mitosis. The appearance of this form could therefore play a role in the activation of *HO*. The other form is found at all stages of the cell cycle. Thus, SBF of one kind or another can be detected throughout the cell cycle, suggesting that the cell cycle regulation of *SWI4* transcription described in [35••] does not cause major fluctuations in SBF concentration during the cell cycle. Swi6 protein, as well as Swi4, is shown to be part of SBF–SCB complexes, which form on three different fragments from URS2. The intracellular location of Swi6 varies during the cell cycle; it accumulates in the cytoplasm of mitotic cells but is concentrated in the nucleus at all other cell cycle stages.

49. ANDREWS BJ, HERSKOWITZ I; **The Yeast SWI4 Protein Contains a Motif Present in Developmental Regulators and is Part of a Complex Involved in Cell-Cycle-Dependent Transcription.** *Nature* 1989, 342:830–833.

50. •• PRIMIG M, SOCKANATHAN S, AUER H, NASMYTH K; **Anatomy of a Transcription Factor Important for the Start of the Cell Cycle in *Saccharomyces cerevisiae*.** *Nature* 1992, 358:593–597.
This paper shows that SBF can be reconstituted by translating *SWI4* and *SWI6* mRNAs in a reticulocyte lysate, that SBF binds to *SCB* DNA via a 110 amino acid domain near the amino terminus of Swi4, and that Swi4 and Swi6 interact via sequences near their carboxyl termini. A version of the DNA-binding domain of Swi4 is found in the *S. pombe* START gene *cdc10*, suggesting that its function is highly conserved.

51. BREEDEN L, NASMYTH K: **Cell Cycle Control of the Yeast *HO* Gene: *cis-* and *trans*-Acting Regulators.** *Cell* 1987, 48:389–397.

52. • MCINTOSH E, ATKINSON T, STORMS R, SMITH M; **Characterization of a Short, *cis*-Acting DNA Sequence Which Conveys Cell Cycle Stage-Dependent Transcription in *Saccharomyces cerevisiae*.** *Mol Cell Biol* 1991, 11:329–337.
This paper describes a thorough analysis of the role of MCB elements in the cell cycle regulation of the *TMP1* promoter.

53. •• NASMYTH K, DIRICK L: **The Role of SWI4 and SWI6 in the Activity of G1 Cyclins in Yeast.** *Cell* 1991, 66:995–1013.
This paper shows that transcription and function of the G_1 cyclin genes *CLN1* and *CLN2* need *SWI4* and *SWI6*, that SBF binds to SCBs within the *CLN2* promoter, and that the lethality of *swi4 swi6* double mutants can be rescued by expression of *CLN2* from a foreign promoter. Thus, SBF is a START-dependent activator not only of *HO*, but also of *CLN1* and *CLN2*.

54. MOLL T, DIRICK L, AUER H, BONKOVSKY J, NASMYTH K: **SWI6 is a Regulatory Subunit of Two Different Cell Cycle-Dependent Transcription Factors in *Saccharomyces cerevisiae*.** *J Cell Sci* 1992, 16 (suppl): in press.

55. ANDREWS BJ, HERSKOWITZ I: **Identification of a DNA Binding Factor Involved in Cell-Cycle Control of the Yeast *HO* Gene.** *Cell* 1989, 57:21–29.

56. •• DIRICK L, NASMYTH K: **Positive Feedback in the Activation of G1 Cyclins in Yeast.** *Nature* 1991, 351:754–757.
To reconcile the Cdc28-dependence of *HO* activation by SBF with it also being an activator of G1 cyclins, which are themselves activators of Cdc28, it was proposed that activation of *CLN1* and *CLN2* by SBF occurs via a positive feedback loop. As predicted by this hypothesis, *CLN1* and *CLN2* transcription is shown to depend on *CDC28* and on G_1 cyclin activity.

57. •• BREEDEN L, MIKESELL GE; **Cell Cycle-Specific Expression of the SWI4 Transcription Factor is Required for the Cell Cycle Regulation of HO Transcription.** *Genes Dev* 1991, 5:1183–1190.
SW4 transcripts fluctuate during the cell cycle, being most abundant in late G_1 at the time of *HO* activation and least abundant in early G_1 and G_2, when *HO* is not transcribed. How important this regulation is for confirming SBF activity to late G_1 and S phase is not clear. SBF-binding activity does not vary much during the cell cycle (see [25]). High-level expression of *SWI4* from the *GAL* promoter seems to cause *HO* to be expressed in G_2, but this effect could be at least partly due to *SWI6* becoming redundant for *HO* transcription under these conditions.

58. • MARINI NJ, REED SI: **Direct Induction of G1-Specific Transcripts Following Reactivation of the Cdc28 Kinase in the Absence of *de novo* Protein Synthesis.** *Genes Dev* 1992, 6:557–567.
The activation of *HO* and DNA replication genes as cells undergo START depends on an active Cdc28 protein kinase. This paper analyses the activation of these genes as temperature-sensitive *cdc28* mutant cells arrested in G_1 due to incubation at the restrictive temperature are allowed to enter the cell cycle following a return to the permissive temperature. Gene activation still partly occurs even when protein synthesis is inhibited by cyclohexamide, implying that the SBF and MBF transcription factors are activated by a post-translational mechanism. However, activation is noticeably delayed by cyclohexamide and the experiments do not rule out another role for *de novo* protein synthesis.

59. ROWLEY A, SINGER RA, JOHNSTON GC; ***CDC68*, a Yeast Gene that Affects Regulation of Cell Proliferation and Transcription, Encodes a Protein with a Highly Acidic Carboxyl Terminus.** *Mol Cell Biol* 1991, 11:5718–5726.

60. REED SI: **The Selection of *S. cerevisiae* Mutants Defective in the Start Event of Cell Division.** *Genetics* 1980, 95:561–577.

61. SUTTON A, IMMANUEL D, ARNDT KT: **The SIT4 Protein Phosphatase Functions in Late G1 for Progression into S Phase.** *Mol Cell Biol* 1991, 11:2133–2148.

62. •• FERNANDEZ-SARABIA MU, SUTTON A, ZHONG T, ARNDT KT: **SIT4 Protein Phosphatase is Required for the Normal Accumulation of *SWI4*, *CLN1*, *CLN2*, and *HCS26* RNAs during Late G1.** *Genes Dev* 1992, 6:2417–2428.
Double mutants carrying a temperature-sensitive *sit4* phosphatase allele and a mutant *ssd1* allele arrest as unbudded G_1 cells at the restrictive temperature. The sudden appearance of *CLN1*, *CLN2* and *HCS26* transcripts as cells undergo START is both delayed and reduced in *sit4 ssd1* double mutants, which might explain their G_1 arrest. Expression of *CLN2* from a foreign promoter allows the double mutant cells to undergo DNA replication but does not restore budding, implying that *SIT4* is required not only for S phase entry but also for bud formation. Ectopic expression of *SWI4* rescues the lethality of certain *cln sit4* double mutants, suggesting that *SWI4* transcription may be one of the defects of *sit4* mutants.

63. NASMYTH K, DIRICK L, SURANA U, AMON A, CVRCKOVA F: **Some Facts and Thoughts on Cell Cycle Control in Yeast.** *Cold Spring Harb Symp Quant Biol* 1991, 56:9–20.

64. CROSS F, ROBERTS J, WEINTRAUB H: **Simple and Complex Cell Cycles.** *Annu Rev Cell Biol* 1989, 5:341–395.

65. LORINCZ A, CARTER BLA; **Control of Cell Size at Bud Initiation in *Saccharomyces cerevisiae*.** *J Gen Microbiol* 1979, 113:287–295.

66. •• BARONI MD, MONTI P, MARCONI G, ALBERGHINA L: **cAMP-Mediated Increase in the Critical Cell Size Required for the G1 to S Transition in *Saccharomyces cerevisiae*.** *Exp Cell Res* 1992, 201:299–306.
It has been known for some time that slow-growing cells undergo START at a smaller size than fast-growing cells (see [65]). This paper shows that the addition of cAMP to mutants carrying a defective cAMP phosphodiesterase has the same effect as shifting cells from poor to rich media, i.e. a temporary inhibition of START until cells grow larger. The implication is that cAMP may be an inhibitor of START and could mediate the control of cell size by nutrients. This is a radical and interesting proposal because much of the earlier literature has emphasized an opposite dependence, i.e. a requirement for cAMP.

67. BARONI MD, MARTEGANI E, MONTI P, ALBERGHINA L: **Cell Size Modulation by *CDC25* and *RAS2* Genes in *Saccharomyces cerevisiae*.** *Mol Cell Biol* 1989, 9:2715–2723.

68. ERREDE B, GARTNER A, ZHAO-QUING Z, NASMYTH K, AMMERER G: **A Pheromone Induced Kinase Cascade in *S. cerevisiae*. Activation of the Fus3 Kinase by the Ste7 Kinase *in vitro*.** *Nature* 1993, in press.

69. CHANG F, HERSKOWITZ I; **Identification of a Gene Necessary for Cell Cycle Arrest by a Negative Growth Factor of**

Yeast: FAR1 is an Inhibitor of a G1 Cyclin, CLN2. *Cell* 1990, 63:999–1011.

70. VALDIVIESO MH, SUGIMOTO K, JAHNG K-Y, FERNANDES P, WITTENBERG C: **FAR1 is Required for Posttranscriptional Regulation of *CLNL* Gene Expression in Response to Mating Pheromone.** *Mol Cell Biol* 1993, **13**:1013–1022.

71. GOEBL MG, YOCHEM J, JENTSCH S, MCGRATH JP, VARSHAVSKY A, BYERS B: **The Yeast Cell Cycle Gene *CDC34* Encodes a Ubiquitin-Conjugating Enzyme.** *Science* 1988, **241**:1331–1335.

72. YOCHEM J, BYERS B: **Structural Comparison of the Yeast Cell Division Cycle Gene *CDC4* and a Related Pseudogene.** *J Mol Biol* 1987, **195**:233–245.

73. HOLLINGSWORTH REJ, SCLAFANI RA: **DNA Metabolism Gene *CDC7* from Yeast Encodes a Serine (Threonine) Protein Kinase.** *Proc Natl Acad Sci USA* 1990, **87**:6272–6276.

74. •• BELL SP, STILLMAN B: **ATP-Dependent Recognition of Eukaryotic Origins of DNA Replication by a Multiplication Complex.** *Nature* 1992, **357**:128–134.
Much more is now known about regulation of the cell cycle than is known about events that are regulated: that is, we know more about the various forms of the Cdc28 protein kinase than we do about its physiological targets. Initiation of DNA replication is one of the major events dependent on Cdc28. This paper describes the identification of a complex that binds to replication origins. Components of this complex and proteins that associate with it could prove to be important substrates for Cdc28.

75. JOHNSTON LH, LOWNDES NF; **Cell Cycle Control of DNA Synthesis in Budding Yeast.** *Nucleic Acids Res* 1992, **20**:2403–2410.

76. •• GHIARA JB, RICHARDSON HE, SUGIMOTO K, HENZE M, LEW DJ, WITTENBERG C, REED SI: **A Cyclin B Homolog in *S. cerevisiae*: Chronic Activation of the Cdc28 Protein Kinase by Cyclin Prevents Exit from Mitosis.** *Cell* 1991, **65**:163–174.
This paper describes the identification of a B-type cyclin gene (*CLB1*) in *S. cerevisiae* that is expressed in G_2. Studies on *Xenopus* extracts (see [30,85]) suggested that the metaphase to anaphase transition depends on the destruction of B-type cyclins. This paper describes the expression of an amino-terminal truncation of Clb1 that causes mitotic arrest. It is suggested that cells arrest in metaphase. In a separate study [79] the arrest caused by stabilized Clb1 and Clb2 proteins was found to be late telophase; this result is inconsistent with the notion that Clb destruction is needed for anaphase.

77. •• AMON A, SURANA U, MUROFF I, NASMYTH K; **Regulation of p34CDC28 Tyrosine Phosphorylation is not Required for Entry into Mitosis in *S. cerevisiae*.** *Nature* 1992, **355**:368–371.
Phosphorylation of Tyr15 in *S. pombe* Cdc2 and the equivalent residues in animal Cdc2 proteins plays an important part in delaying the onset of mitosis, particularly when DNA replication is inhibited. Phosphorylation of the equivalent tyrosine residue in Cdc28 is cell cycle regulated but it plays little role in the G_2 arrest of cells whose DNA is unreplicated or damaged. Other mechanisms must therefore exist for regulating entry into M phase which might not even involve regulation of Cdc28.

78. GRANDIN N, REED SI: **Differential Function and Expression of *Saccharomyces cerevisiae* B-Type Cyclins in Mitosis and Meiosis.** *Mol Cell Biol* 1993, in press.

79. SURANA U, AMON A, DOWZER G, MCGREW J, BYERS B, NASMYTH K: **Destruction of the CDC28/CLB Mitotic Kinase is not Required for the Metaphase/Anaphase Transition in Budding Yeast.** *EMBO J* 1993, in press.

80. LYDALL D, AMMERER G, NASMYTH K: **A New Role for MCM1 in Yeast: Cell Cycle Regulation of *SW15* Transcription.** *Genes Dev* 1991, **5**:2405–2419.

81. BENDER A, SPRAGUE GF: **MATa1 Protein, a Yeast Transcription Activator Binds Synergistically with a Second Protein to a Set of Cell Type Specific Genes.** *Cell* 1987, **50**:681–691.

82. WEINERT TA, HARTWELL LH: **The *RAD9* Gene Controls the Cell Cycle Response to DNA Damage in *Saccharomyces cerevisiae*.** *Science* 1988, **241**:317–322.

83. •• SORGER PK, MURRAY AW; **S-Phase Feedback Control in Budding Yeast is Independent of Tyrosine Phosphorylation of p34cdc28.** *Nature* 1992, **355**:365–368.
This paper reports similar results to those in [77••].

84. RUSSELL P, MORENO S, REED SI: **Conservation of Mitotic Controls in Fission and Budding Yeasts.** *Cell* 1989, **57**:295–303.

85. GLOTZER M, MURRAY AW, KIRSCHNER MW; **Cyclin is Degraded by the Ubiquitin Pathway.** *Nature* 1991, **349**:132–138.

86. NASMYTH K, ADOLF G, LYDALL D, SEDDON A; **The Identification of a Second Cell Cycle Control on the *HO* Promoter in Yeast: Cell Cycle Regulation of SWI5 Nuclear Entry.** *Cell* 1990, **62**:631–647.

87. •• MOLL T, TEBB G, SURANA U, ROBITSCH H, NASMYTH K; **The Role of Phosphorylation and the Cdc28 Protein Kinase in Cell Cycle-Regulated Nuclear Import of the *S. cerevisiae* Transcription Factor Swi5.** *Cell* 1991, **66**:743–758.
The cell cycle regulation of the entry of Swi5 into the nucleus is shown to depend on phosphorylation sites within or near the Swi5 nuclear localization signal. It is proposed that destruction of the Cdc28 protein kinase at the end of mitosis causes Swi5 dephosphorylation and thereby activation of its nuclear localization signal. Regulated Swi5 nuclear entry is not necessary for mother cell specific *HO* expression but probably regulates other genes, which unlike *HO*, are expressed as soon as cells enter G_1.

88. STILLMAN DJ, BANKIER AT, SEDDON A, GROENHOUT G, NASMYTH KA; **Characterization of a Transcription Factor Involved in Mother Cell Specific Transcription of the Yeast *HO* Gene.** *EMBO J* 1988, 7:485–494.

89. •• DOHRMANN PR, BUTLER G, TAMAI K, DORLAND S, GREENE JR, THIELE DJ, STILLMAN DJ; **Parallel Pathways of Gene Regulation: Homologous Regulators SWI5 and ACE2 Differentially Control Transcription of *HO* and *chitinase*.** *Genes Dev* 1992, 6:93–104.
ACE2 encodes a protein that is closely related to the product of *SWI5* and is required for the activation of the *CTS1* chitinase gene but not for *HO*. Ace2 and Swi5 have very similar DNA-binding domains and nuclear localization signals. Furthermore, their synthesis and entry into the nuclear are similarly cell cycle regulated. The paper suggested that *CTS1* is also dependent on *SWI4* and *SWI6* and that its regulation is therefore similar to that of *HO*. These last two assertions may be incorrect because *CTS1* is transcribed normally in *swi4* and *swi6* mutants and, unlike *HO*, is activated as soon as cells enter G_1, due presumably to the regulated entry of *Ace2* into the nucleus (R Siegmund, T Schuster, K Nasmyth, unpublished data).

90. HARTWELL LH, WEINERT TA: **Checkpoints: Controls that Ensure the Order of Cell Cycle Events.** *Science* 1989, 246:629–634.

91. WINEY M, GOETSCH L, BAUM P, BYERS B: ***MPS1* and *MPS2*: Novel Yeast Genes Defining Distinct Steps of Spindle Pole Body Duplication.** *J Cell Biol* 1991, **114**:745–754.

92. •• HOYT MA, TROTIS L, ROBERTS BT: ***S. cerevisiae* Genes Required for Cell Cycle Arrest in Response to Loss of Microtubule Function.** *Cell* 1991, 66:507–517.
Treatment of cells with microtubule inhibitors causes cells to arrest cell division in a state with high Cdc28 protein kinase activity. This paper (along with [93••]) describes the isolation of *bub* mutants that fail to arrest in this manner, which were identified by virtue of their hypersensitivity to a sublethal inhibitor dose. The mutant cells continue DNA replication in the absence of microtubule function but cannot complete cytokinesis. It is suggested that the *BUB* genes are needed to transmit the state of a cell's microtubules to regulators of mitosis and that cell cycle arrest might be due to a delay in degradation of the Cdc28 kinase. Like *RAD9*, which is needed for DNA damage induced cell cycle arrest, *BUB2* is a non-essential gene.

93. •• LI R, MURRAY AW: **Feedback Control of Mitosis in Budding Yeast.** *Cell* 1991, 66:519–531.
This paper describes the isolation of *mad* mutants that have a similar phenotype to *bub* mutants. *MAD2* is, however, an essential gene.

94. KIMELMAN D, KIRSCHNER M, SCHERSON T: **The Events of the Midblastula Transition in *Xenopus* are Regulated by Changes in the Cell Cycle.** *Cell* 1987, **48**:399–407.

95. LEW DJ, REED SI: **A Proliferation of Cyclins.** *Trends Cell Biol* 1992, **2**:77–81.

96. KOFF A, GIORDANO A, DESAI D, YAMASHITA K, HARPER JW, ELLEDGE S, NISHIMOTO T, MORGAN DO, FRANZA BR, ROBERTS JM: **Formation and Activation of a Cyclin E–cdk2 Complex During the G1 Phase of the Human Cell Cycle.** *Science* 1992, **257**:1689–1694.

97. PAGANO M, DRAETTA G, JANSEN-DÜRR P: **Association of cdk2 Kinase with the Transcription Factor E2F during S Phase.** *Science* 1992, **255**:1144–1147.

98. NURSE P, BISSETT Y; **Gene Required for Commitment to Cell Cycle and in G2 for Control of Mitosis in Fission Yeast.** *Nature* 1981, **292**:448–460.

99. HAGAN I, HAYLES J, NURSE P: **Cloning and Sequencing the Cyclin-Related *cdc13*$^+$ Gene and a Cytological Study of its Role in Fission Yeast Mitosis.** *J Cell Sci* 1988, **91**:587–596.

100. •• BUENO A, RICHARDSON H, REED SI, RUSSEL P: **A Fission Yeast B-Type Cyclin Functioning Early in the Cell Cycle.** *Cell* 1991, **66**:149–159.

This paper describes the isolation of a second type of B-type cyclin gene in *S. pombe* called *cig1*. Deletion of *cig1* is not lethal but causes a marked delay in the onset of DNA replication.

101. FORSBURG SL, NURSE P: **Identification of a G1-Type Cyclin puc1$^+$ in the Fission Yeast *Schizosaccharomyces pombe*.** *Nature* 1991, **351**:245–248.

102. •• LOWNDES NF, MCINERNY CJ, JOHNSON AL, FANTES PA, JOHNSTON LH: **Control of DNA Synthesis Genes in Fission Yeast by the Cell-Cycle Gene *cdc10*$^+$.** *Nature* 1992, **355**:449–453.

The *cdc10* gene is required for the initiation of DNA replication in *S. pombe* [4]. A clue as to its function stemmed from the discovery that *cdc10* is extensively homologous to *SWI6* from *S. cerevisiae* [45], which encodes one of the components of the late G_1-specific SBF transcription factor. This paper shows that the cdc10 protein is a component of a factor from *S. pombe* tht binds MCB elements. Swi6 is also a component of an equivalent factor in *S. cerevisiae* (MBF/DSC1 [46••,47••]). The activation of gene expression at the beginning of the cell cycle is therefore a conserved feature of the cell cycle.

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