The pharmacology of cell differentiation
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SWITCHING GENES ON AT THE START OF THE YEAST CELL CYCLE

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INTRODUCTION

It was the discovery that DNA replication occurs at a discrete stage during interphase that led to the division of the eukaryotic cell cycle into the now familiar G1, S, G2, and M phases (1). We now recognize that G1 can be subdivided into early and late phases. Early G1 cells can either embark on a new cell cycle or enter a quiescent state or undergo differentiation. Later in G1, cells become committed to DNA replication and often also to the completion of mitosis. In the budding yeast S. cerevisiae, the transition from early to late phases of G1 is known as Start (2). The equivalent process in mammalian cells has been called the restriction point (3). In both yeast and mammalian cells, the Start transition requires protein synthesis and growth to a critical cell size. What is the physical basis behind Start? What proteins need to be synthesised and what determines the timing of their synthesis? How does their synthesis lead to the onset of S phase?

START INVOLVES THE ACTIVATION OF THE CDC28 PROTEIN KINASE BY CYCLINS

The analysis of temperature sensitive mutants that arrest at specific stages of the cell cycle identified 50 or more cell division cycle (*CDC*) genes. Of these, only *CDC28* (4) and *CDC37* (5) are required for Start (see figure 1). CDC28 encodes a highly conserved protein kinase, which is homologous to the cdc2 kinase of fission yeast and mammalian cells (6). The function of *CDC37* remains unkown. One of the striking aspects of the cdc2/CDC28 kinase is that it is required not only for Start but also for mitosis, at least in fungi. Slightly different isoforms of the kinase, cdk2 and cdc2, seem to perform the two tasks in mammalian cells (7).

Insight into how the cdc2/CDC28 kinase can be involved in such diverse functions as DNA replication and mitosis originated with the discovery that the kinase is only active when complexed with a class of proteins called cyclins (8). There are many different types of cyclins which have been discovered by diverse means. A and B type cyclins were discovered due to cell cycle dependent oscillations in their abundance during sea urchin cleavage divisions (9), whereas the CLN3 cyclin was discovered because mutants that stabilize it cause yeast cells to undergo Start with an abnormally small cell size (10, 11). All cyclins share a conserved domain of120 amino acids. Most are unstable and most, though not all, oscillate in abundance during the cell cycle. The genetic analysis of cyclin function is most advanced in

the yeast Saccharomyces cerevisiae. Here, one of three G1-specific cyclins encoded by *CLN1, 2,* and *3* are required for Start and DNA replication (12), whereas B-type cyclins encoded by *CLB1, 2, 3,* and *4* are involved in the formation and function of the mitotic spindle (13) (see figure2).

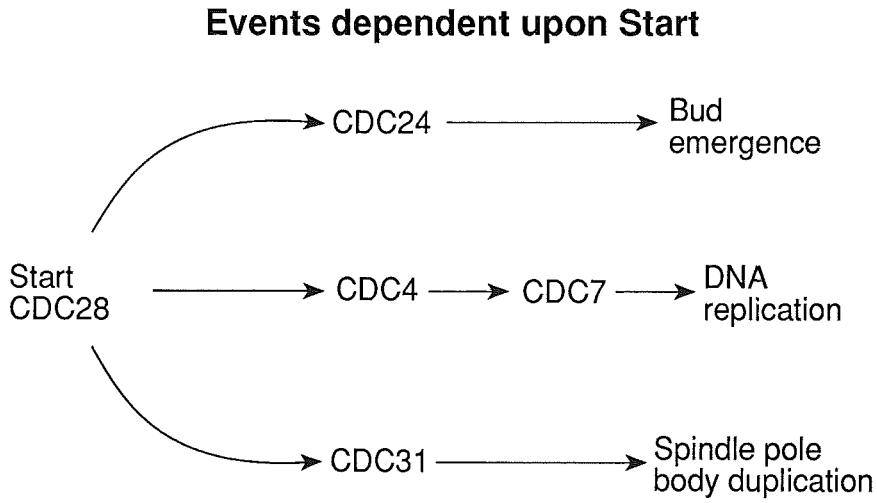


Figure1. Three different events follow the start of the cell cycle in yeast.: Bud emergence, DNA replication, and Spindle pole body duplication. All three are dependent on the *CDC28* gene. The other genes shown have more specific functions. *cdc28* mutant cells are still capable of mating when arrested at the restrictiive temperature; i.e. they are not yet committed to the mitotic pathway.

CDC28 can be isolated as an active kinase when associated with CLN (15) or CLB cyclins (16). In all but one case, the CDC28 kinase activity associated with yeast cyclins is cell cycle dependent. The CLN1 and CLN2 associated kinase appears when cells undergo Start and dissappears when they enter G2 (17), whereas CLB2 associated kinase appears during G2, peaks just prior to anaphase, and dissappears as cells enter G1 (18). The one exception is CLN3, whose protein levels and associated CDC28 kinase do not vary much during the cell cycle (17). It is currently thought that the appearance of a CLN/CDC28 kinase triggers cells to undergo Start (17), that the appearance of CLB/CDC28 kinase triggers the onset of mitosis (14), and that the destruction of CLB/CDC28 kinase triggers the exit from mitosis and re-entry into G1(18). cdc2/CDC28 kinase activity is not only regulated by changes in cyclin abundance, which clearly play a key part in the initiation and end of the cell cycle, but also by changes in the phosphorylation of the cdc2/CDC28 protein, which in many animal cells and the fission yeast S. pombe plays a key part in the timing of mitosis (19).

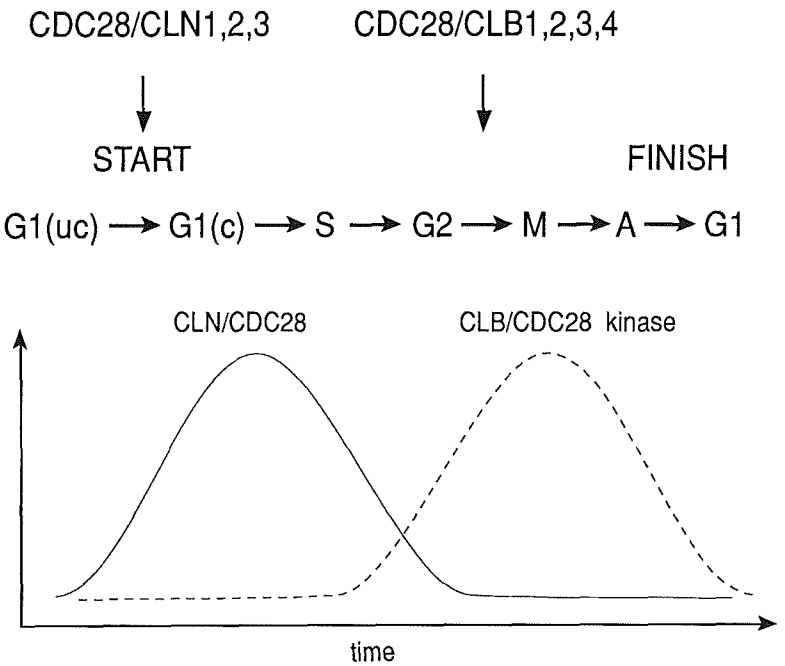


Figure 2. *CDC28* is required not only for S phase but also for mitosis. One of three G1 cyclins encoded by *CLN1, 2, 3* is required for Start whereas four B-type cyclins encoded by *CLB1, 2, 3, 4* are involved in mitosis. The CDC28 kinase is only active when complexed to a cyclin. The G1 period is divided into uncommitted (uc) and committed (c) phases.

The sudden activation of CLN/CDC28 kinase is sufficient to drive an early G1 cell immediately through Start (17). Whether a sudden increase in CDC28 kinase is necessary for this process is however less clear. Though greatly delayed, Start can still occur in mutants lacking *CLN1* and *CLN2* (12). Under these circumstances, Start is dependent on CLN3, which does not appear to vary during the cell cycle, at least of wild type cells (17). Does this mean that Start can be triggered without any sudden change in CLN/CDC28 kinase? There is some evidence that CLN3 is not sufficient for Start and that some as yet unidentified cyclin must also be activated (see below).

Transcriptional regulation of *CLN1* and *CLN2* plays an important, if not vital, part in the timing of Start during yeast proliferation. Both transcripts are absent in small early G1 cells but appear suddenly around the time of Start (20, 21). Moreover, unscheduled expression of either gene from the *GAL* promoter greatly accelerates the entry of cells into S phase (17). What leads to the sudden activation of *CLN1*

and *CLN2* in late G1? What is known about transcription factors whose activity may be specific to late G1 cells?

G1 CYCLINS ARE ACTIVATED BY THE SWI4/SWI6 TRANSCRIPTION FACTOR

SWI4 and SWI6 were discovered as transcription factors needed for the activation of the *HO* gene, which encodes an endonuclease that induces mating type switching (22, 23). *HO* was the first example of a large family of genes, including those for G1 cyclins and most DNA replication enzymes, that are activated at Start (24). Activation of this gene family is dependent on the CDC28 protein kinase but, unlike histone genes, is not dependent on other genes required for entry into S phase like *CDC4* (see figure 1). An important reason for studying *HO* regulation was the hope that it would prove to be a useful tool for investigating the function of *CDC28*; *HO* activation seemed a good biochemical marker for Start.

The choice of *HO* to study cell cycle transcription factors was a fortunate one because *HO*'s dispensability encouraged the search for its transcription factors by genetic means; this is a task that is not lightly undertaken if it is known beforehand that the genes whose regulation is being studied are essential for cell cycle progression, as would have been the case for DNA replication genes. In practise, the analyis of *HO* cell cycle regulation was complicated by *HO* being regulated not only by cell cycle stage but also by a cell's history; i.e whether it is a mother or daughter cell. As a consequence, there are at least two modes of cell cycle regulation acting on *HO*, one restricting its expression to G1 (20) and a second further constraining this window to late G1 (22). In retrospect, it is perhaps not surprising that more than a ten factors (encoded by *SWI1-10*) are required for *HO* activation and and an additional four genes are required for its correct regulation (22).

The singling out of SWI4 and SWI6 as factors specifically concerned with a *CDC28* or Start dependent activation pathway relied on the prior identification of a sequence element, now known as the SCB (Swi Cell cycle Box), that is sufficient for confering this aspect of *HO* regulation (25). Deletion of the region of the *HO* promoter containing SCBs causes *HO* expression during G1 to become simultaneously independent of *CDC28*, *SWI4* and *SWI6* without affecting the dependence on all eight other SWI genes (26). In addition, the transcriptional activation of a reporter gene by SCB elements alone was shown to be dependent on *CDC28*, *SWI4*, and *SWI6* but not on other SWI genes (26). We now know that SWI4 and SWI6 are two components of a factor called SBF (SCB Binding Factor) that binds to multiple SCB elements (27, 28). SBF can be detected in crude yeast extracts using a gel retardation assay (28, 29) and it can be re-constitued by co-translation of SWI4 and SWI6 in a reticulocyte lysate (30). The SCB is recognized by 120 amino acid DNA binding domain at the N terminus of the SWI4 protein. Unlike SWI4, SWI6 cannot bind SCB DNA on its own in vitro but it can be recruited into ternary complexes due to the interaction of SWI4 and SWI6 via their C terminal sequences (see figure 3). Mutations that remove SWI4's C-terminal sequences

prevent it from activating *HO* (Table 1 and ref. 31). Surprisingly, when we first cloned the *SWI4* gene, we isolated a truncated version capable of activating *HO* when expressed from a centromere containing plasmid. It transpires that SWI4 is over-expressed from this plasmid and this allows it to function in the absence of SWI6. Overexpression of the full length SWI4 protein from the *GAL* promoter also allows *HO* to be expressed in the absence of SWI6 (31, 32). The simplest interpretation of these results is that SWI6 normally facilitates the binding of SWI4 to SCBs but this function becomes redundant if SWI4's concentration is raised.

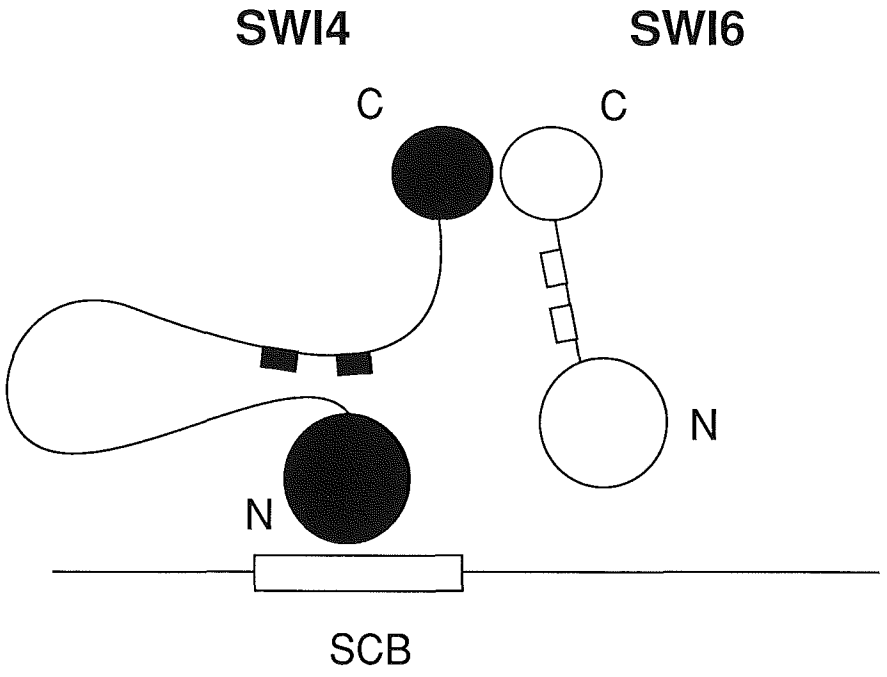


Figure 3. A rough model for the SBF transcription factor composed of SWI4 and SWI6. SWI4 alone can bind to a single SCB but it is less clear whether the SWI4/SWI6 complex can do so. It may need multiple SCBs to bind. The small boxes represent TPLH motifs. SWI6 contains a leucine zipper that could have a role in multimerization.

Although SWI4 and SWI6 have unequal roles in SBF, there are indications that they may have evolved from a common ancestor. The central regions of the two proteins are homologous, both containing two copies of a 34 amino acid motif first discovered in SWI6 (33), since found in a very wide variety of eukaryotic proteins (34), and now called the TPLH or ANK repeat. The TPLH motif has been implicated in protein-protein and in one case at least in protein-DNA interactions (35). The role of these motifs in SWI4 and SWI6 is not yet understood. Mutations

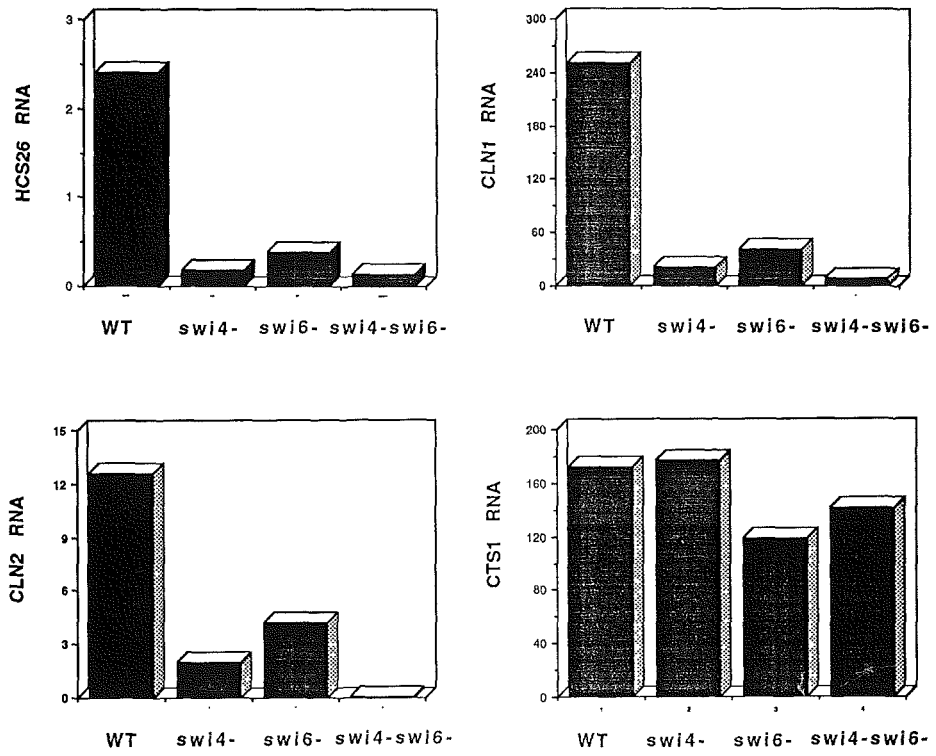


Figure 4. The level of *CLN1*, *CLN2*, *HCS26*, and *CTS1* transcripts in *swi* mutants. All strains were congenic with K1107 (38) and all contained a single copy of *CLN2* expressed from the S.pombe *ADH* promoter. This has no effect on transcription from endogenous *CLN* genes It ameliorates the growth defect of *swi4* and *swi6* single mutants and allows the *swi4 swi6* double mutant to grow.

in SWI4's first repeat prevent it from activating *HO*. The mutant protein is stable but cannot form ternary complexes on SCBs with SWI6 when co-translated in vitro nor can mutant protein over-expressed from yeast bind SCBs.

It is still unclear how the activity of SBF is regulated through the cell cycle. *SWI4* transcripts are most abundant in late G1, which raises the possibility that changes in *SWI4* transcription itself could play a part in the regulating *HO* (32). However, there are no large fluctuations in the level of SBF binding activity during the cell cycle (28), suggesting that SWI4 protein, at least when complexed with SWI6, may be quite stable. The variation in *SWI4* transcription could still cause modest (up to two fold) changes in the concentration of SBF, but it seems unlikely that this would be sufficient to regulate *HO*, unless SBF newly synthesised in G1 behaved differently from SBF inherited from the previous cell cycle. *SWI4* expression from the *GAL* promoter throughout the cell cycle does disturb the repression of *HO* in G2 (32), but this also causes *HO* to become independent of *SWI6*, which is likely to be the primary cause of the de-regulation (36).

There is some evidence that post-transcriptional events could regulate SBF activity. Cells arrested in early G1 due to the incubation of a ts *cdc28* mutant at the restrictive temperature express the *HO* gene soon after being returned to the permissive temperature. If a G1 cyclin is overproduced, then *HO* can still be partly activated in the absence of protein synthesis (37). A change in the electrophoretic proterties of SBF at the time of *HO* activation has been detected using a gel retardation assay but this change persists throughout the rest of the cell cycle (28). If the change is related to the formation of an active form of SBF, then repression during G2 must occur by a process that is not simply a reversal of the activation. The best working hypothesis at present is that either SWI4 or SWI6 is a substrate for the CDC28 kinase and that phosphorylation is responsible for SBF's activation. SWI6 changes its localization during the cell cycle. It is concentrated in the nucleus for most of the cell cycle but much accumulates in the cytoplasm in mitotic cells (28). This could play some role in the repression of SBF during G2 but is unlikely to be important for its activation at Start because SWI6 is found concentrated in the nucleus as soon as cells enter G1.

Though neither SWI4 nor SWI6 are essential genes in haploid yeast strains, the deletion of both genes causes lethality, implying that they have functions in addition to the activation of *HO* (26). It is now clear that one of these functions is the activation of the G1 cyclin genes *CLN1* and *CLN2* (38, 39). Both genes are poorly transcribed in *swi4* or *swi6* mutants and their expression from a moderately active foreign promoter is sufficient to rescue the double mutant (38). The importance of SWI4 and SWI6 for G1 cyclin transcription in wild type cells has been best estimated by measuring the level of *CLN1* and *CLN2* RNAs transcribed from their endogenous promoters in cells that also express *CLN2* ectopically (see figure 4); *SWI4* deletion causes a severe drop whereas*SWI6* deletion has a more modest effect, (40). The decreases are, however, less severe than at *HO*, whose transcription is virtually absent in *swi4* or *swi6* single mutants (26). Both *CLN1* and *CLN2* must therefore also be activated weakly by an activator other than SBF. The data also suggest that, unlike *HO*, *CLN1* and *CLN2* can be partially activated by

A Paradox: CLN3 function is dependent on SWI 4,6 though its transcription is not.

Genotype	Growth phenotype	Conclusion
cln1Δ cln2Δ CLN3	+	G1 cyclin CLN3
cln1Δ cln2Δ cln3Δ	–	has a START function
swi4Δ swi6Δ	–	insufficient G1 cyclins are active in *swi4 swi6* double mutants
swi4Δ swi6Δ pADH - CLN2	+	
cln1Δ cln2Δ CLN3 swi4Δ	–	The START function of CLN3 is dependent on SWI4 and SWI6
cln1Δ cln2Δ CLN3 swi6Δ	-	

Hypothesis:

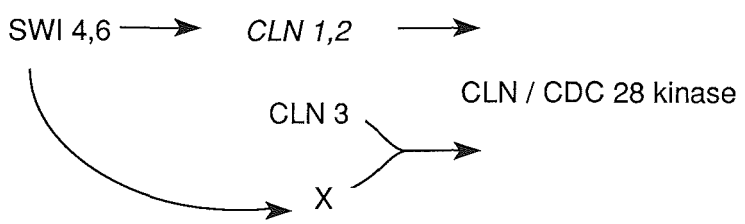


Figure 5. CLN3 may not be sufficient for Start. Unlike *CLN1* and *CLN2*, *CLN3* transcription is not dependent on *SWI4* or *SWI6*.

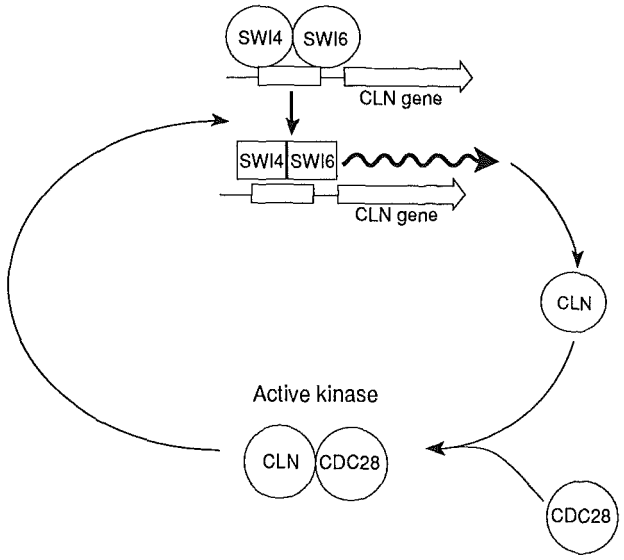
SWI4 acting without SWI6. However, under these circumstances neither gene is as tightly cell cycle regulated as in wild type (36). Another function of SBF is the activation of the *HCS26* gene, which encodes a distant member of the cyclin family that is expressed in late G1 (figure 4 and ref39). SBF probably activates *CLN2* and *HCS26* directly by binding to SCB elements in their promoters. The promoters of both these genes contain SCB-like sequences to which SBF can bind (38, 39), but in neither case has the function of these binding sites been tested by mutation. All the genes dependent on SBF show *CDC28* dependent activation in G1. The *CTS1* gene, which encodes a chitinase involved in cell separation, has been reported also to depend on *SWI4* and *SWI6* (41). This is a surprising result because, according to our data, *CTS1* is expressed in early G1 (R. Siegmund, personal communication). We have therefore re-investigated the *CTS1* dependence on *SWI4* and *SWI6* and find no evidence of any effect (figure 4).

The lethality of *swi4 swi6* double mutants cannot be simply explained by their failure to express *CLN1* and *CLN2*. The deletion of any pair of the three *CLN* genes is not lethal but the deletion of all three causes cells to arrest in early G1. This suggested that any one of the *CLN* genes might be sufficient for Start (12). *CLN3* transcription occurs normally in *swi4 swi6* double mutants but in this instance it is not sufficient to drive cells through Start, unlike ectopic expression of *CLN2* (38). The CLN2 and CLN3 proteins clearly do not in fact have equivalent functions; CLN3 protein requires *SWI4* and *SWI6* to promote Start but CLN2 protein does not. The simplest explanation for these results is that, in the absence of *CLN1* and *CLN2*, *CLN3* cannot drive cells through Start without the help of some factor, which might be a fourth as yet unidentified G1 cyclin, whose transcription is dependent on *SWI4* and *SWI6* (see figure 5). The unknown factor is not just HCS26 because the simultaneous deletion of *CLN1*, *CLN2*, and *HCS26* is not lethal (Fatima Cvrckova; personal communication).

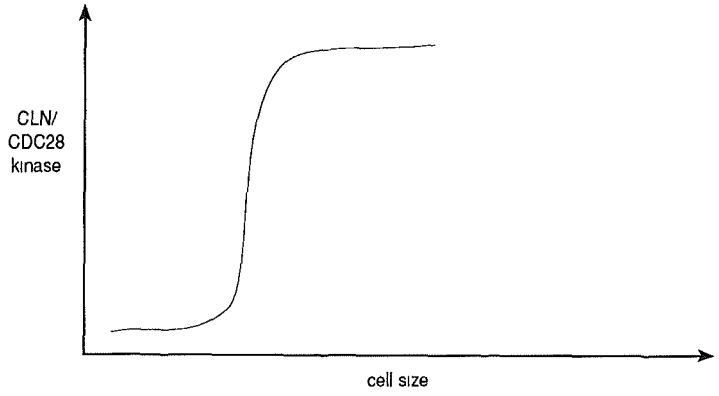
STRAIN	BETA-GLACTOSIDASE UNITS
WT	202
swi4::*URA3* TPN	0.8
SWI4 TPN	199
swi4 STP1 TPN	10
swi4 SPT2 TPN	18
swi4 STP3 TPN	8
swi4 STP4 TPN	14

TABLE 1. Truncations of SWI4 cannot activate *ho* transcription when transplaced to the *SWI4* locus. Nonsense codons at nucleotide positions 2442 (STP1), 2608 (STP2), 2803 (STP3), 3084 (STP4), relative to the *SWI4* AUG codon, were introduced to the *SWI4* locus by a two step gene transplacement procedure (TPN). The *swi4* gene was first replaced by the *URA3* gene and this was later replaced by *SWI4* mutants. LacZ activity from an *ho* LacZ fusion was measured.

CLNs may promote their synthesis via SWI4,6



(+)ve feedback may facilitate all or none CLN/CDC28 kinase activation and hence irreversibility of START



How the switch from Low to high CLN expression is triggered by cell size is unknown.

Figure 6. CLN proteins promote their own synthesis by activating the CDC28 protein kinase, which is required for the SWI4/SWI6 transcription factor SBF to be fully active. How SBF is activated by CDC28 is not yet understood.

START MAY INVOLVE THE ACTIVATION OF A POSITIVE FEEDBACK LOOP

The discovery that SBF is required for the activation of *CLN1* and *CLN2* brought to light an important paradox. SBF activity at *HO* needs an active CDC28 kinase, which in turn requires the expression of *CLN1* and *CLN2*; i.e. SBF is not only dependent on the CDC28 kinase but also an activator of it. There are two explanations for this paradox: either there are two modes of action of SBF, one that is *CDC28*- independent and involved in the activation of G1 cyclins and a second that is *CDC28*-dependent and involved in the activation of *HO*, or SBF and G1 cyclin activation occurs via a positive feedback loop through which kinase activates SBF, which activates G1 cyclin transcription, which closes the loop by activating kinase. As predicted by the latter hypothesis, *CLN1* and *CLN2* transcription is dependent on *CDC28* and on G1 cyclin activity and can be triggered by the ectopic expression of any one G1 cyclin gene (42, 43). In its simplest form the positive feedback would involve the activation of SBF by phosphorylation of SWI4 or SWI6 by the CDC28 kinase. This property has not yet been demonstrated.

A positive feedback loop may help explain the apparent irreversibility of Start. It is to be expected that the SBF/G1 cyclin regulatory circuit would have only two stable states: one with low kinase and a second with high kinase (see figure 6). It seems likely that the transition from the low to the high kinase state forms the biochemical basis to Start. This event normally only occurs when cells reach a critical cell size. 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 weak mechanisms for cyclin activation that are not dependent on the pre-existence of kinase activity and that the strength of this activation pathway is somehow proportional to cell size or to the cell's protein synthetic capacity. The CLN3 cyclin, which does not oscillate much during the cell cycle (17), could play an important role in this process. Another key question concerns how G1 cyclin transcription is later repressed as cells enter G2.

A PAIR OF START DEPENDENT TRANSCRIPTION FACTORS

The group of genes regulated by SBF are not alone in being activated in late G1 (see figure 7). Most if not all genes encoding enzymes involved in DNA replication are also transcribed only transiently during the cell cycle, as cells undergo Start (reviewed in ref 44). The promoters of these genes all contain one or more copies of a sequence motif whose core is an MluI restriction site (45). In several cases, these MluI sequences have been shown to be both necessary for the activity of promoters containing them and sufficient to confer cell cycle regulation on a reporter gene (46, 47). The motif is therefore now known as the MCB (MluI Cell cycle Box).

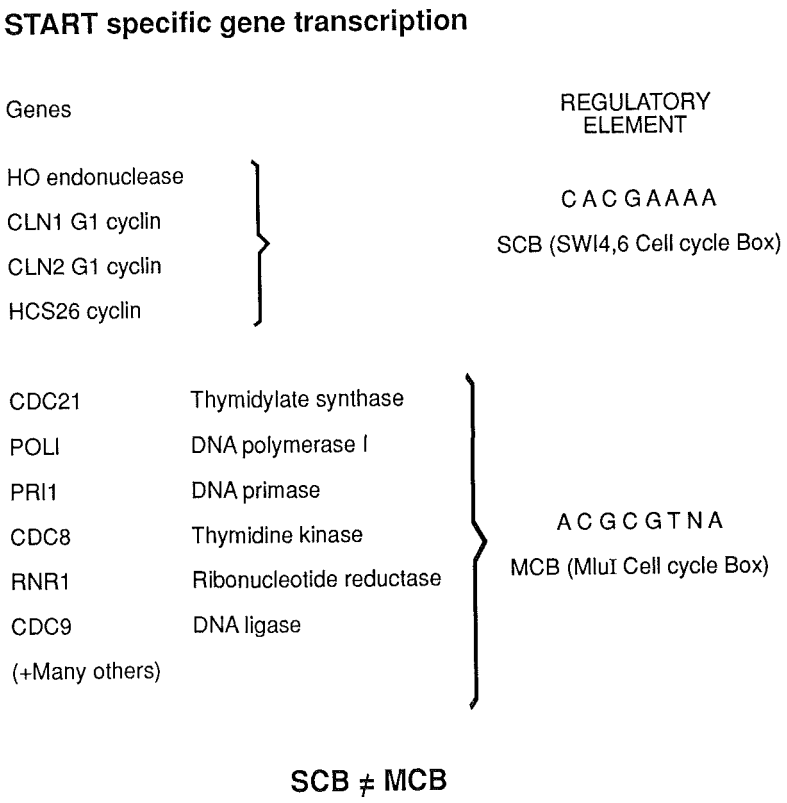


Figure 7. There are two groups of genes activated at Start.: those whose promoters contain SCB elements and are depedent on SBF (top) and others whose promoters contain MCB elements and are probably activated by MBF (bottom). Most genes involved in DNA replication are activated by MCB elements.

The fact that *swi4* deletion mutants are viable but *swi4 swi6* double mutants are not suggests that SWI6 has a function in addition to its role in the biological activity of SCB elements. SWI6 cannot bind to SCB elements at all in the absence of SWI4 and the greater severity of the double mutant phenotype cannot therefore be due to residual activation at SCBs by SWI6 alone. Might SWI6's additional role concern the activity of MCB elements? We have purified a binding activity (MBF) that has different chromatographical properties to SBF and is capable of recognizing a pair of natural MCB elements from the *TMP1* (thymidylate synthase) promoter. MBF may be the same as an activity called DSC1 (48) that binds to tandemized MluI sites (the MCB is more than just an MluI sites because they alone do not confer late G1-specific transcription). Like SBF, MBF seems to be composed of at least two

proteins, one of which is SWI6 and the other a 120 kd (p120) protein that is not SWI4. UV cross-linking studies suggest that the major contacts between MBF and MCB DNA are made via P120.

There is almost no change in the abundance of MCB regulated transcripts due to the deletion of *SWI6* but there is a profound change in their regulation and, in the case of the *RNR1* ribonucleotide reductase gene, a change in the site of transcription initiation (36 and 49). Instead of a sharp peak in transcript abundance in late G1, the transcripts are equally abundant throughout the cell cycle. One explanation for this phenotype is that p120, like SWI4 at *CLN2*, can bind MCBs and at least partially activate transcription in the absence of SWI6, but under these circumstances it is no longer responsive to cell cycle control (see figure 8). Surprisingly, SCB elements can compete with the binding of MBF to MCBs. It has therefore been proposed that the p120 protein, whose gene has not yet been identified, is related to SWI4 (36). Thus, SBF and MBF may be related not only because they share a SWI6 subunit but also because the DNA binding subunits of each factor, SWI4 and p120, may be related proteins.

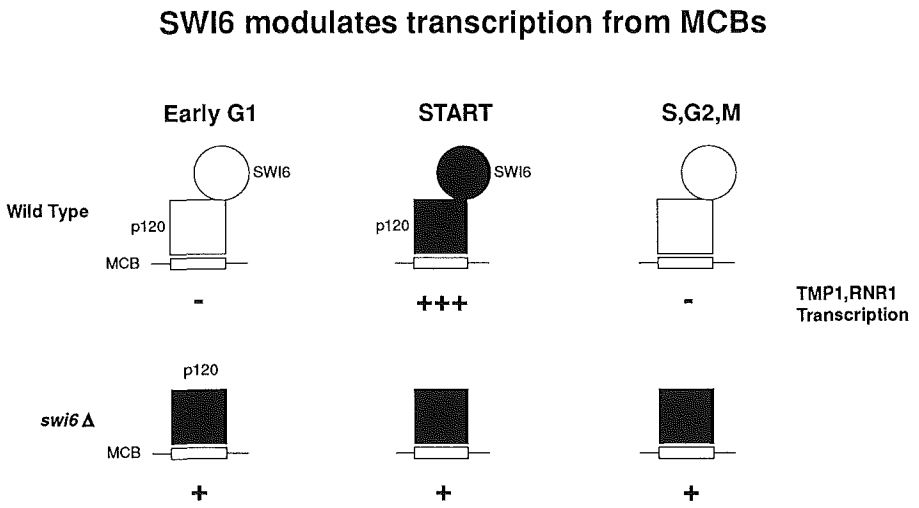


Figure 8. A hypothesis to explain why MCB dependent genes, such as *TMP1* and *RNR1*) are no longer cell cycle regulated in *swi6* mutants It is proposed that p120 (squares) can bind to MCBs and activate transcription in the absence of SWI6 (circles) but it is now no longer responive to cell cycle control.

CONCLUSIONS

This article began with the question of what happens inside a yeast cell when it undergoes Start. Until recently, the prevailing view was that transcriptional

controls would not be an important aspect. One reason for this view was that it was known that cleavage embryos can go through many cell cycles without any transcription. The second reason was the observation that most yeast cell cycle gene products are present in excess and do not need to be re-synthesised each cell cycle for further cell cycle progression (50). It is now clear that cell cycle specific gene activation is vital for the entry into and progression through the mitotic programme of a yeast cell. G1 and G2-specific cyclins must be transcribed at Start and in G2 respectively.

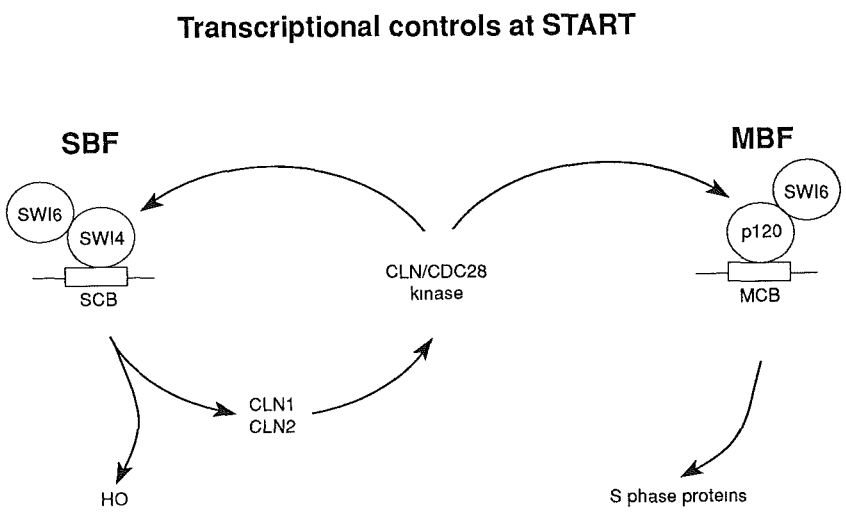


Figure 9.

Two classes of genes are activated at Start (see figure 9). The first includes *HO*, *CLN1*, *CLN2*, and *HCS26*, which are activated via the binding of the SBF transcription factor to SCB elements. The second includes most genes involved in DNA replication, which are probably activated via the binding of the MBF or DSC1 transcription factor to MCB elements. SBF and MBF are related factors whose regulation is similar and which share a common regulatory subunit, SWI6. The activation of G1 cyclins by SBF occurs via a positive feedback loop and is an important feature of Start. The role of the MBF activated transcription for cell cycle progression is less clear because most of the genes regulated by MBF encode stable enzymes that are present in considerable excess. This by no means excludes the possibility that MBF also regulates genes whose products are unstable and are required for S phase. Thus, gene activation by MBF could well play an important part in S phase.

An obvious question is whether the transcriptional programme regulated by SBF and MBF is a conserved feature of the eukaryotic cell cycle. The *cdc10* gene from the fission yeast Schizosaccharomyces pombe is required for Start (51), encodes a protein homologous to SWI6 (33), and is part of an MBF or DSC1-like factor that binds to MCBs (52). It thus seems that the SBF and MBF transcription factors may be conserved amongst fungi. G1 cyclins have also been discovered in mammalian cells (53). Cyclin E transcripts appear in late G1 at around the time of the restriction point but nothing is yet known about the transcription factor responsible for this regulation. So far, no protein homologous to SWI4 or SWI6 has been discovered in mammalian cells.

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Discussion - SWITCHING GENES ON AT THE START OF THE YEAST CELL CYCLE

H.F. Lodish

Are yeast cyclins turning over? Are they physically unstable as they are in early oocytes? That is, are the proteins themselves turning over?

K. Nasmyth

Yes, in all cases so far looked at, all of the proteins are unstable. Now that is not to say that all of the cyclins have been analyzed at that level, but when they have been analyzed they have been found to be unstable. In addition, when analyzed, all have been shown to cycle, except for one. One of the G_1 cyclins, a G_1 cyclin called GLB 3, is probably just as unstable as any other cyclin, but as far as we know it doesn't cycle during the cell cycle and this led to considerable confusion, because the initial analysis of the G_1 cyclin suggested that GLB 3, which doesn't cycle, was sufficient to get herself through Start. So, how could you get a cell going through Start on a cyclin that didn't cycle?

H.F. Lodish

What do you mean it doesn't cycle? Do you mean that its level is constant?

K. Nasmyth

The protein appears to be constant throughout the cell cycle and the kinase activity associated with CDC 28 appears to be fairly constant throughout the cell cycle, so this is really a major problem. Now, we are beginning to have some clues as to what is going on: when one does a more sophisticated analysis of what the role of this cyclin is, it appears that GLB 3 could well not be sufficient to get a cell through Start and that in fact it needs other cyclins, possibly including GLB 5. So, at present, the notion is that GLB 3 is a sort of start. Because there is this positive feedback loop, the cyclins have got to turn themselves on, it would be nice to have a system that will trigger this. Kinase activity is needed to turn on G_1 cyclin activity, and at some stage

you have got to start with some kinase and GLB 3 may be the starter kinase. I think it is interesting possibly to draw a parallel between GLB 3 in Saccharomyces and possibly the cyclin D's of mammalian cells. Those seem not to cycle terribly dramatically, but there seems to be considerable consensus that cyclin E is a late G_1, it is a cyclin that really does cycle. Cyclin D's turn on when you stimulate quiescent cells but it is not so clear that they turn off in a cell cycle-dependent manner in quite the same way as other truly cell cycle regulated cyclins. It is just conceivable that cyclin D could be the functional homologue to GLB 3, but it is a little bit provocative to say that.

J. Massagué

Along the same lines, activity is not only defined by, or dependent on, the level of cyclin protein but also the set of events that post-translationally activate the cyclin-CDK complex. Do you have evidence for phosphorylation or other events that might activate the kinase activity associated with these G_1 cyclins?

K. Nasmyth

That is a very important point. So far there is no evidence for any post-transcriptional regulation of G_1 cyclin activity. If you take an early G_1 cell, a very small G_1 cell that shouldn't undergo Start for several hours, and you then simply turn on G_1 cyclins like CLN 1 or CLN 2, that is sufficient to send a cell immediately through Start. So the cell doesn't have to wait for anything else, and indeed if you express G_1 cyclins at any stage in the cell cycle you can produce G_1 associated kinase. So at present it appears that the way of regulating G_1 kinase is through transcription, at least in budding yeast. So far, there is no evidence, with regard to the normal passage of cells through the cell cycle, that they are regulated by post-transcriptional events.

One aspect of the yeast cell cycle I didn't touch on, is the fact that pheromones can shut down passage through Start, and it is clear that the pheromones must inactivate the G_1 cyclins. There the evidence seems to indicate that the pheromones do so by a post-transcriptional mechanism. They can inhibit G_1 kinase activity through the fab 1 protein and this is acting directly on the kinase, although it may also act on

transcription. With regard to the size control, that is the fact that the G_1 cyclins turn on at a particular stage in the cell cycle, there is no evidence of any post-transcriptional control, but in the case of pheromone control there is evidence of post-transcriptional regulation.

J. Massagué

Naïvely, one would think that there ought not to be a need for a second layer of regulation on top of the transcription control. Transcriptional regulation alone with subsequent production of the protein ought to be sufficient to provide the function performed by this protein. Why should there be so much additional regulation built into the G_2 M cyclins? Why should they be inactive as a kinase complex, and dependent on phosphorylations and dephosphorylations for activation? What role do you think all of this plays? Why wouldn't it be enough to have just transcription of cyclin B's after the mitotic cyclins in order to complete the cell cycle?

K. Nasmyth

It is extremely important, for example, that G_2 cyclins should not become active too soon in the cell cycle, otherwise the cell cycle would be short circuited. One of the functions of keeping the kinase could be just to prevent that. One of the things that has become very clear over the last five or ten years of yeast work, and that is now becoming clear from work with mice where one can also knock out genes, is that there is enormous redundancy in all these things and that it is not just good enough to have a cell that will just limp through the cell cycle, but you want one that will do it with extreme efficiency and one which can do it competitively, and I think that probably 9/10's of all genes are concerned with competitive advantage as opposed to just getting through life. It is interesting to compare G_2 with G_1 control. In the G_1 control it is the appearance of cyclins or the activity of G_1 cyclins that is regulated, and in the case of G_2 control it is the catalytic activity of the CDC 28 or CDC 2 kinase itself. Ultimately, I think we should be able to understand why one event is regulated by regulating cyclin activity and the other event seems in many instances to be regulated by regulating the kinase itself.

G.E. Francis

Referring to this sort of second layer of regulation, isn't it possible that it is there to cope with events like DNA damage requiring a slow down or block of entry into S?

K. Nasmyth

That certainly is correct in some instances, but it doesn't have to be like that because in the budding yeast Saccharomyces cerevisiae, what you're referring to is the phosphorylation on the tyrosine residue in CDC 2, which seems to play an important role in preventing premature entry into mitosis, for example in the absence of DNA replication being completed. Budding yeast cells also have elaborate mechanisms for preventing that, but this tyrosine residue, as far as we can tell at the moment, plays no role in that control. So in order to regulate mitosis you do not have to do it through the CDC 2 kinase.

S.H. Friend

It seems as if you had differences in the regulation of this MCB and SCB box containing genes. If, Swi6 is a part of both of those complexes and Swi6 is regulating that activity in at least one other box that is cell cycle dependent, how is it that you can put Swi6 in both of those complexes and only see the cell cycle, or at least the G_2 cyclin, affecting one of those two boxes?

K. Nasmyth

It looks like the Swi4/Swi6 transcription factor is turned off by G_2 cyclins, but the MBP Swi6 transcription factor is not. Something else is turning it off. What they both have in common is Swi6 and what is different between these two is Swi4 versus MBP. I think what this is telling us is that the target of the G_2 cyclins on the Swi4/Swi6 transcription factor is not Swi6, because that is common to both proteins, but it would seem to suggest that the target is in fact Swi4.

M. Crescenzi

Concerning the role of transcriptional versus post-translation control in cell

cycle, is there any evidence, in yeast, that by turning on one or more genes the rest of the cell cycle machinery follows as a consequence of having activated those genes?

K. Nasmyth

If you take a very small cell that shouldn't undergo Start for several hours, because it hasn't yet achieved a critical rate of protein synthesis and if you just turn on the transcription of a G_1 cyclin, that will immediately send that cell into S phase. So merely turning on the transcription of a G_1 cyclin will immediately trigger the whole cell cycle programme.

M. Oren

You mentioned that all those players, the Swi 4/6 and MBP have ANK repeats. Is there any evidence that there is any translocation upon activation of those proteins, anything to do with retaining it in cytoplasm versus nucleus?

K. Nasmyth

In the case of Swi6 there is some evidence that the entry into the nucleus is under control of the cell cycle. What you find is that Swi6 is concentrated in the nucleus throughout the G_1 period, so it is there in early G_1 and late G_1. This says that translocation, of Swi6 at least, is playing no role in the activation of these genes at Start, because it is in the nucleus before Start and after Start. But during G_2, when cells go into mitosis, much of the Swi5/Swi6 protein is found in the cytoplasm. Not all of it, but we now know that Swi6 is not homogeneous, it is bound to two different factors, one is Swi4 and the other is MBP, and there may be a third one as far as we know. So, when we are looking at Swi6 we have to take into account that we may be looking at several different proteins. Nonetheless, a lot of the protein appears in the cytoplasm and then when the cells exit from mitosis then suddenly it becomes concentrated in the nucleus again. It is just conceivable that the cytoplasmic accumulation of Swi6 plays some role in shutting off transcription of these genes in G_2, but the entry into the nucleus cannot be playing an important role in triggering transcription in G_1 and it is not known whether the ANK repeats within Swi6 have any function in this cytological control.

M. Oren

It is interesting that they are really conserved to a very great extent between your MBP and Swi4, so it's really like they are fulfilling an essential function.

K. Nasmyth

Yes, it seems they are fulfilling a very important function. I think, in fact, that all these - Swi4, Swi6, MBP - are related proteins and that they all descend from a common ancestor. So far there is no evidence for mammalian Swi4, MBP's and Swi6's, but what we do know is that there are very related genes in the fish and yeast S. bombé, where the CDC10 gene is very homologous to Swi6 and clearly is part of a MBF-like transcription factor. These transcription factors are very conserved amongst fungi and that usually means they will be conserved among eucaryotes, because the rate of evolution of fungi is enormously higher than that of animals, but so far there's no hint of such factors in animals.