Review Article

Starting to cycle: G1 controls regulating cell division in budding yeast

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Introduction

The yeast *Saccharomyces cerevisiae* divides by a process that is atypical of eukaryotes; the mother cell produces a small bud that enlarges and ultimately separates as the daughter cell. Nevertheless, many aspects of cellular organization, structure and function in this yeast are also found in multi-cellular organisms. Coupled with the advantages of a small genome and amenability to genetic manipulation (see for example Broach *et al.*, 1991), this conservation has made *S. cerevisiae* a useful model system for the analysis of many eukaryotic processes. One particular example is the control of cell growth and division for which *S. cerevisiae* and the distantly related fission yeast *Schizosaccharomyces pombe* have been used extensively (reviewed in Forsburg & Nurse, 1991). Identification of many genes whose products are exclusively involved in cell division, the cloning of several of these genes and the characterization of their products has shed light on how progress through the cell cycle is controlled at the molecular level. Accumulated experimental evidence has resulted in the formulation of an integrated model of how *S. cerevisiae* controls its mitotic cell cycle, which has wider implications for cell cycle control in eukaryotes in general.

In *S. cerevisiae*, as in all eukaryotes, the mitotic cell cycle can be divided into four intervals, G1-, S-, G2- and M-phase. S-phase and M-phase are the periods when DNA replication and nuclear division occur respectively, and overall control of cell division is achieved by regulating entry into these two phases. The major controlling event in *S. cerevisiae*, termed START, occurs late in G1 (Pringle & Hartwell, 1981). Completion of START requires that a cell grows beyond a minimum size and commits the cell to a round of mitotic division

as opposed to alternative developmental fates such as conjugation or, for diploid cells, meiosis. Commitment to mitotic division sets in train the pathways required for the initiation of DNA synthesis and the transition to S-phase.

Entry into both S- and M-phases requires the activation of a 34 kDa protein serine/threonine kinase encoded by the *CDC28* gene which is functionally equivalent to the *cdc2*⁺ gene product of *Schiz. pombe* (Reed *et al.*, 1985; Reed & Wittenberg, 1990; see Nurse, 1990). The enzymic activity of both p34^{CDC28} and p34^{cdc2} is regulated at least in part by assembly of the kinase subunit into a complex with members of a family of labile proteins, the G1 and mitotic cyclins (see Forsburg & Nurse, 1991, and references therein). Functional conservation of the key elements in this complex in virtually all higher eukaryotes from plants to man (Nurse, 1990) has led to the conclusion that the essential features of the mitotic cell cycle have been highly conserved throughout evolution.

In this review, we will concentrate specifically on the controls that operate during the G1-phase of the *S. cerevisiae* cell cycle. We will describe the molecular mechanisms which act to control p34^{CDC28} protein kinase activity at START, and examine the ways in which the cell responds to this activation in order to progress from G1- to S-phase. Other recent reviews have focussed on specific aspects of this topic (Nurse, 1990; Nasmyth, 1990; Johnston & Lowndes, 1992; McKinney & Cross, 1992; Lew & Reed, 1992).

The concept of START

Much of our current understanding of cell cycle control in *S. cerevisiae* comes from studies of cells carrying conditional-lethal mutations in cell division cycle (*CDC*) genes (reviewed in Broach *et al.*, 1991). Amongst this

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Table 1. Genes whose products have a known or putative role in cell cycle progress during G1 in budding yeast

Gene	Gene product
(a) START A	
<i>CDC25</i>	Positive regulator of <i>RAS</i> activity
<i>CDC35</i>	Adenylate cyclase
<i>RAS1</i>	Positive regulator of adenylate cyclase
<i>RAS2</i>	Positive regulator of adenylate cyclase
<i>CDC33</i>	Protein synthesis initiation factor 4E (eIF4E)
<i>CDC60</i>	Leucyl-tRNA synthetase
<i>IRA1</i>	Negative regulator of <i>RAS</i> activity
<i>IRA2</i>	Negative regulator of <i>RAS</i> activity
<i>BCY1</i>	cAMP-dependent protein kinase regulatory subunit
<i>TPK1</i>	cAMP-dependent protein kinase catalytic subunit
<i>TPK2</i>	cAMP-dependent protein kinase catalytic subunit
<i>TPK3</i>	cAMP-dependent protein kinase catalytic subunit
<i>SCH9</i>	Protein kinase
<i>YAK1</i>	Protein kinase
<i>SIT4</i>	Protein phosphatase
<i>PRT1</i>	Protein synthesis initiation factor 3 subunit (eIF3)
(b) Signal transduction	
<i>STE2</i>	α -factor receptor
<i>STE3</i>	a-factor receptor
<i>SCG1/GPA1</i>	G protein α subunit
<i>STE4</i>	G protein β subunit
<i>STE18</i>	G protein γ subunit
<i>STE20</i>	Protein kinase
<i>STE5</i>	?
<i>STE11</i>	Protein kinase
<i>STE7</i>	Protein kinase
<i>STE12</i>	Transcription factor
<i>KSS1</i>	Protein kinase
<i>FUS3</i>	Protein kinase
<i>FAR1</i>	Inhibitor of G1 cyclin function
<i>CDC36</i>	Negative transcriptional regulator
<i>CDC39</i>	Negative transcriptional regulator
(c) START B	
<i>CLN1</i>	G1 cyclin; Cdc28 kinase regulatory subunit
<i>CLN2</i>	G1 cyclin; Cdc28 kinase regulatory subunit
<i>CLN3</i>	G1 cyclin; Cdc28 kinase regulatory subunit
<i>ORFD</i>	Putative G1 cyclin
<i>CLB5</i>	B type cyclin with putative G1 function
<i>HCS26</i>	Putative G1 cyclin
<i>SSX1</i>	? Regulator of <i>SWI4</i> transcription
<i>SWI4</i>	Component of SBF transcription factor
<i>SWI6</i>	Component of SBF and DSC1 transcription factors
?	p120; component of DSC1 transcription factor
<i>CDC28</i>	Protein kinase catalytic subunit
(d) Post-START	
<i>CDC4</i>	?
<i>DBF4</i>	Cdc7 kinase regulatory subunit
<i>CDC7</i>	Protein kinase catalytic subunit

collection of mutants are some whose products are required late in G1-phase and which, under restrictive conditions, arrest the cell cycle at START. These can be further subdivided on the basis of the morphology of the arrested cells. In START A mutants, (Baroni *et al.*, 1992; formerly START class II), this morphology resembles that of cells arrested under conditions of limited nutrient availability. In contrast, in START B mutants (formerly

START class I) the morphology is characteristic of cells that have arrested in response to treatment with mating pheromone. START A gene functions precede, and are necessary to execute, START B. In this section we will examine the pathways defined by START A and START B genes, before moving on to consider the possible networking between them, and the post-START events that lead to the initiation of DNA synthesis. A list of the genes and gene products involved in these events is given in Table 1.

Functional interactions governing START

START A

Progress through START A requires functioning of the *RAS*-adenylate cyclase pathway, which regulates the production of cyclic AMP (cAMP). In budding yeast, the proteins encoded by the *RAS1* and *RAS2* genes are required to maintain the activity of adenylate cyclase, encoded by *CDC35*, at levels sufficient to overcome the hydrolysis of cAMP by phosphodiesterase (Nikawa *et al.*, 1987). The Ras proteins themselves serve as a focal point for the regulation of adenylate cyclase, being positively regulated by the *CDC25* gene product which promotes GDP/GTP exchange on the Ras proteins and inhibited by interaction with the products of the *IRA1* and *IRA2* genes. Deletion of *CDC25* is lethal, implying a role for the Cdc25 protein in maintaining the basal level of Ras activity, but deletion of both *IRA1* and *IRA2* from the yeast genome suppresses *cdc25* null mutants and increases Ras activity (Tanaka *et al.*, 1989, 1990).

The ultimate target of the *RAS*-adenylate cyclase pathway is cAMP-dependent protein kinase (cAMP-PK), also called protein kinase A. In its inactive form, cAMP-PK is a tetramer comprising two catalytic subunits encoded by the functionally redundant genes, *TPK1*, *TPK2* and *TPK3*, bound to two regulatory subunits encoded by *BCY1* (Toda *et al.*, 1987a, b; Cannon *et al.*, 1990). Production of cAMP by the *RAS*-adenylate cyclase pathway activates cAMP-PK, probably by promoting the dissociation of the regulatory and catalytic subunits (Thevelein, 1991, 1992; Hirimburegama *et al.*, 1992), hence releasing the catalytic subunit to act on components downstream (Fig. 1).

Temperature sensitive mutants for cAMP synthesis arrest at START A at the restrictive temperature, whereas mutants which have enhanced levels of either cAMP or cAMP-dependent protein kinase activity are incapable of arrest at this point even under conditions of limited nutrient availability. It was therefore postulated that an adequate nutritional status is signalled to adenylate cyclase by the *RAS* gene products (Gibbs & Marshall, 1989; Dumont *et al.*, 1989). This is supported

Fig. 1. A model for the signal transduction pathways of START A in yeast. Activation of cAMP-dependent protein kinase can occur either via the *RAS*-adenylate cyclase pathway (upper part of Figure) or via the nitrogen-induced pathway (lower part). Arrows indicate activation, blocked lines indicate down-regulation and broken arrows indicate a putative pathway linking START A to START B through the Sit4 protein phosphatase.

by the observation that addition of fermentable sugar to derepressed yeast cells causes a rapid peak in the cAMP level which appears to initiate a protein phosphorylation cascade (François *et al.*, 1984).

Recent data indicate that cellular detection of adequate levels of nitrogen, sulphate and phosphate is dependent on cAMP-PK activity directly rather than on cAMP production itself (Hirimburegama *et al.*, 1992). An elegant model to explain these data was proposed by Thevelein (1992) in which a nitrogen/sulphate/phosphate-induced pathway is able to activate free (and only free) catalytic subunits of cAMP-dependent protein kinase. Hence, cAMP-dependent protein kinase, rather than cAMP itself, appears to be the universal integrator of nutrient availability. Mutants of the nitrogen signalling pathway include *CDC33*, which encodes the protein synthesis initiation factor 4E (Brenner *et al.*, 1988) and *CDC60*, which encodes the cytosolic leucyl-tRNA synthetase (Hohmann & Thevelein, 1992). Furthermore, temperature sensitive mutants of isoleucyl- and methionyl-tRNA synthetase also arrest at START A (Unger & Hartwell, 1976), as do mutants of *PRT1*, which encodes a subunit of eIF3 (Linder & Prat, 1990). The ability to synthesize proteins is thus incorporated into the nutrient signalling pathway (Fig. 1).

Recent experiments have demonstrated that sugar induced activation of the *RAS*-adenylate cyclase path-

way is repressed by glucose (Beullens *et al.*, 1988; Mbonyi *et al.*, 1990; Argüelles *et al.*, 1990), and is therefore not normally active during exponential growth using glucose as a carbon source. Thevelein (1991) proposed that in glucose repressed cells the nitrogen signalling pathway, which is dependent on glucose or another fermentable carbon source, triggers progression through START A. These results at first sight seem difficult to reconcile with the fact that various mutants of the *RAS*-adenylate cyclase pathway arrest irrespective of the growth media. However, repression of the pathway by glucose only eliminates the cAMP signal, whereas mutations in the pathway will also eliminate the basal level of cAMP which is required for activation of protein kinase A (see Thevelein, 1992). The activation of protein kinase A is in turn required for passage past START A which, in the absence of mating pheromone, results in Cdc28 function at START B; this is discussed below.

START B

Regulation of *CDC28* activity. The completion of START B is critically dependent on activation of the *CDC28* gene product, p34^{CDC28}, and a comprehensive understanding of START has entailed a detailed analysis of the molecular mechanisms which regulate p34^{CDC28} activity during G1. The major mechanism by which the

protein kinase activity of this protein is regulated at both mitosis and START is by periodic association with specific regulatory proteins termed cyclins (Mendenhall *et al.*, 1987). In *S. cerevisiae*, these cyclins can be characterized on the basis of their ability to activate p34^{CDC28} kinase either at mitosis (mitotic cyclins) or at START (G1 cyclins). Six genes encoding putative G1 cyclins have been isolated from budding yeast, of which three, *CLN1*, *CLN2* and *CLN3* (formerly *DAF1* or *WHI1*), have been extensively characterized and shown to comprise a functionally redundant gene family, since cells containing inactivated alleles of any two *CLN* genes, though displaying a growth phenotype, are still able to traverse START, whilst a triple mutant is inviable (Richardson *et al.*, 1989). These experiments suggest that each *CLN* gene product is capable of carrying out all necessary G1-cyclin functions, although the efficiency with which each protein operates in different pathways may vary (see below).

Despite this functional redundancy, *CLN1* and *CLN2* are regulated transcriptionally in a cell cycle-dependent fashion with maximum transcript levels accumulating in late G1; *CLN3*, in contrast, appears to be constitutively transcribed (Wittenberg *et al.*, 1990). The significance of this transcriptional difference between the three genes is not clear (Lew *et al.*, 1992, 1993; Linskens *et al.*, 1993), since all three proteins contain specific amino acid motifs near their C-termini (PEST sequences), which are believed to confer inherent instability on proteins and which may serve as the signal for the rapid degradation of *CLN* gene products after the onset of DNA replication (Wittenberg *et al.*, 1990), offering another level of regulation for cyclin activity. Despite the differences in their regulation, both *CLN2* and *CLN3* gene products have been shown to interact directly with p34^{CDC28} to form a complex in which p34^{CDC28} is active as a protein kinase (Wittenberg *et al.*, 1990; Tyers *et al.*, 1992). It seems likely that the Cln1 protein acts in a similar manner, although as yet there is no direct evidence for this.

In addition to the three *CLN* genes, *HCS26*, *ORFD* and *CLB5* also encode putative G1 cyclins whose transcription is periodically regulated. Unlike *CLB5*, which was isolated as a suppressor of lethality in a strain carrying a triple *cln* mutation, *HCS26* is unable to rescue the triple *cln* mutation (Ogas *et al.*, 1991), suggesting that although the Hcs26 protein has some homology to the *CLN* proteins, it can carry out only some of the Cln protein functions and may be required to activate p34^{CDC28} kinase at START for a different subset of functions. However, it remains to be shown that the Hcs26 protein interacts directly with p34^{CDC28}.

Activation of the p34^{CDC28} kinase by association with a G1 cyclin is a major component of START. Amongst the

many putative *in vivo* targets for the p34/G1 cyclin complex that have been identified, two have been extensively characterized with respect to their role in the integration and coordination of events at START. These are the Swi4/Swi6 and DSC1 transcription factors, whose functions are discussed below

G1 cyclin regulation: positive control. The *SWI4* and *SWI6* genes were isolated on the basis of their requirement for START-dependent transcription of the *HO* gene, which encodes a double stranded DNA endonuclease that initiates mating type switching in homothallic strains of yeast (reviewed in Herskowitz, 1989). Although the *HO* gene is not essential, *swi4 swi6* double mutants are inviable, suggesting that their gene products perform some other essential role in cell division (Breedon & Nasmyth, 1987). Experiments have shown that Swi4 and Swi6 together are necessary for transcriptional activation of *CLN1*, *CLN2* and *HCS26* (Nasmyth & Dirick, 1991; Ogas *et al.*, 1991). This activation is mediated at a specific sequence, the Swi4/Swi6 Cell Cycle Box or SCB, by the binding of the transcription factor SBF (SCB-Binding Factor) which contains the *SWI4* and *SWI6* gene products. SCB elements are present in the promoter sequences of *CLN1*, *CLN2*, *HCS26*, *ORFD* and *HO* (Breedon & Nasmyth, 1987; Ogas *et al.*, 1991) although interestingly, this motif is absent from the *CLN3* promoter which is not transcriptionally regulated in the same manner. However, Swi4 and Swi6 proteins are involved in the post-translational regulation of Cln3 activity (Nasmyth & Dirick, 1991). Thus, five putative G1 cyclins are dependent on *SWI4* and *SWI6* for activation at START, whereas *CLB5* may be regulated by the transcription factor DSC1 (see below).

Although transcriptional activation of *CLN1* and *CLN2* requires Swi4 and Swi6 proteins, it is also dependent on functional Cln protein already being present in the cell and it was postulated that the Swi4/Swi6 complex is activated by a p34^{CDC28}/G1 cyclin complex, thus establishing a positive feedback loop (Cross & Tinkelenberg, 1991; Nasmyth & Dirick, 1991; Dirick & Nasmyth, 1991). This model is supported by several observations, notably that Swi6 is a phosphoprotein which contains three copies of the putative p34^{CDC28} kinase target consensus, and that it can be efficiently phosphorylated *in vitro* by immune complexes containing the human homologue of p34^{CDC28} (N. Lowndes, C. Norbury & L. Breedon, unpublished observations, cited in Lowndes *et al.*, 1992a). Switching the p34^{CDC28} loop from a low to a high kinase state could be a biochemical basis of START, while the existence of only two stable states, either on or off, would explain its irreversibility. This model requires that Swi4 and/or

Fig. 2. The signal transduction pathway leading to cell cycle arrest in haploid cells in response to pheromone. The pathway shown is for the binding of α -factor to its receptor, encoded by *STE2*. The pathway in response to α -factor is presumed to be the same after *STE3*-encoded receptor binding. Arrows indicate activation, blocked lines indicate down-regulation.

Swi6 proteins exhibit a Cdc28-independent mode of activation to initiate this loop, and could be the pathway by which growth and cell division are coupled.

G1 cyclin regulation; negative control in response to mating factors. The predominant vegetative form of *S. cerevisiae* is a non-mating diploid which, upon starvation, undergoes meiosis to produce two types of haploid spore, *MATa* and *MAT α* . Haploid cells secrete a peptide factor that prepares cells for mating: *MAT α* cells produce α -factor while *MATa* cells produce α -factor. Mating factors cause cells of the opposite type to arrest in the G1 phase of the cell division cycle at START before initiating DNA synthesis. Cell cycle arrest before DNA synthesis is crucial to maintain the haploid state, so that mating produces a *MATa/MAT α* diploid. The mating factors also stimulate synthesis of proteins required for cell and nuclear fusion. However, the precise sequence of events by which mating factors cause cell cycle arrest is unclear and the role of several gene products in the pathway is unknown or uncertain. Nevertheless, analysis of nonresponsive and constitutive mutants which either cannot mate or else initiate the mating response in the absence of mating factor has suggested a model (Fig. 2) in which the response to mating factor response is channelled into the cell cycle via the G1 cyclins (for recent reviews see Kurjan, 1992; Hirsch & Cross, 1992).

According to this model, the initial event in the mating factor response involves the binding of either α - or α -factor to its specific membrane-bound receptor, which are encoded by the *STE2* and *STE3* genes respectively. Binding of mating factor to its receptor at the extra-cellular face of the cell membrane induces a conformational change that is transmitted to the cytoplasmic face of the receptor (Sprague, 1991). Signal transduction on the cytoplasmic face is mediated through a G protein to which the receptor is coupled. By means of the exchange of GDP for GTP on the α subunit, followed by release of β and γ subunits of the G protein, the signal is relayed to downstream components of the mating pathway. Mutations in the *CDC36* and *CDC39* genes cause cells to activate the mating factor response inappropriately, by action at or near to the G protein. The precise role of the *CDC36* gene product is unknown, while the Cdc39 protein appears to be involved in regulating transcription, probably of genes encoding components of the pathway (Neiman *et al.*, 1990; Collart & Struhl, 1993).

Release of the G-protein $\beta\gamma$ -subunits results in activation of a protein kinase cascade (Fig. 2) within which the Ste5 protein appears to play a structural role. One of the targets of this cascade is the transcription factor encoded by *STE12*, whose activation is correlated with increased phosphorylation (Song *et al.*, 1991). Ste12

Fig. 3. A model for the events of START B and the pathway to DNA synthesis in yeast. We postulate that the Cdc28 protein exists in two pools. One of these is regulated by Cln3 and is responsible *primarily* for activation of the SBF and DSC1 transcription factors (see text). The second pool of Cdc28 responds to Cln1 and/or Cln2 and is *primarily* responsible for the activation of elements that lead to Cdc4 function. Symbols are as described in the legend to Fig. 1.

promotes *FAR1* and *FUS3* expression which leads in turn to cell cycle arrest by inhibition of multiple *CLN* functions. Fus3 also inactivates Ste7 by phosphorylation, thus providing a sensitive feedback mechanism for the regulation of the pheromone response pathway.

Thus although a great deal is known about the genes and interactions which regulate START A and START B, little is known about possible links between the two pathways. The limited data that is available about the integration of START A and START B is discussed in the following section.

Integrating START A and START B

Four genes have been isolated whose products are thought to have a role in START networking, although in the case of two of these genes, *SCH9* and *YAK1*, there is no direct evidence to support this view, which is based largely on their effects on START A mutants (Fig. 1; Toda *et al.*, 1988; Garret & Broach, 1989). Both *SCH9* and *YAK1* encode serine/threonine protein kinases. Sch9 protein kinase is homologous to the TPK-encoded kinase, and indeed its overexpression is able to rescue lethality caused by deletion of the three *TPK* genes which encode the catalytic subunits of cAMP-dependent protein kinase. However, deletion of *SCH9* is not lethal, but rather results in a slower growth rate with an extended G1 phase. Therefore Sch9 may function in concert with

or downstream of cAMP-dependent protein kinase. If it is indeed a downstream component of the signalling pathway to cyclin synthesis, then the non-lethal phenotype of the *SCH9* deletion mutant would suggest that another protein kinase acts in parallel with it. The *YAK1* gene product acts in a negative manner with respect to cAMP-dependent protein kinase function since deletion of *YAK1*, which has no phenotype in a wild-type background, can partially rescue the lethality of a triple *TPK* deletion. Furthermore, *YAK1* overexpression causes arrest only in strains with reduced activity of protein kinase A (Garret *et al.*, 1991). The precise role and execution point of *YAK1* is, however, unclear.

In addition to *SCH9* and *YAK1*, two other genes have been implicated in the integration of START A and START B, both of which have effects on the transcription of *SWI4*. One of these genes, *SIT4*, encodes a protein phosphatase that was originally identified from *sit4* alleles that resulted in increased transcription of the *HIS4* gene in the absence of factors that are normally required for its transcription (Arndt *et al.*, 1989). Sit4 protein phosphatase activity has been shown to be required for the normal cell cycle dependent accumulation of *SWI4*, *CLN1*, *CLN2* and *HCS26* RNAs during late G1 (Fernandez-Sarabia *et al.*, 1992). Although the actual substrates for Sit4 protein phosphatase activity *in vivo* are not known, the role of Sit4 in the periodic accumulation of the G1 cyclins is probably at least partly

via Swi4, and a simple model (Fernandez-Sarabia *et al.*, 1992) proposes that Sit4 induces *SWI4* transcription which results in the transcription of the G1 cyclins, as discussed above. However, the situation is further complicated by the observation that some *sit4* mutations have variable, gene-specific effects on transcription and are associated with changes in the transcription of a number of genes, implying that one of the substrates of Sit4 may be an RNA polymerase. If this were the case then Sit4 function may not be directly involved in the cell cycle, but might activate the transcription of a particular subset of genes, some of which may be involved in the cell cycle. The timing of this activation by Sit4 may be mediated by the periodic associations that the Sit4 protein undergoes with two proteins, p155 and p190 (Fig. 3), to form two separate complexes which persist from late G1 to about mid or late mitosis (Sutton *et al.*, 1991). The regulation of these associations may be controlled by post-translational modification of p155 and p190, which are both phosphorylated *in vivo*.

The fourth gene involved in networking START A and START B is *SSX1*, which is defined by recessive mutations that deregulate the periodic expression of *SWI4*, causing its constitutive transcription (Breedon & Mikesell, 1991). The product of *SSX1* appears to act as a negative regulator of *SWI4* transcription that must be inactivated at the appropriate time in the cell cycle to allow *SWI4* expression late in G1. Whether *SWI4* expression is directly influenced by Ssx1, or whether the Ssx1 protein acts further upstream in the pathway that induces *SWI4* expression is unknown. However, the periodic expression of *SWI4* (Breedon & Mikesell, 1991), coupled with periodic nuclear localization of Swi6 during G1 and S-phases (Rita *et al.*, 1991) may therefore provide a mechanism by which initial SBF activity can be regulated, allowing cells to undergo START B and activate the functions needed for progress through G1 to S phase (Fig. 3).

Coordinate expression of genes required for DNA synthesis

The expression of a number of genes whose products are required for DNA synthesis is restricted to late G1 and early S phases of the cell cycle (for a review see Merrill *et al.*, 1992). This group comprises at least 17 genes, the products of which include components of the enzyme machinery for DNA synthesis as well as enzymes involved in the biosynthesis of precursors (Table 2). The coordinate expression of these genes, with maximum transcript levels at the G1–S phase boundary, is probably associated with mechanisms to ensure the correct timing of the initiation of DNA synthesis within the cell cycle.

The coordinate expression of the genes listed in Table

1 is achieved in response to a specific base sequence within the promoter region. This motif, termed MCB (*Mlu*I Cell Cycle Box) as it contains the recognition sequence for the restriction endonuclease *Mlu*I, is the binding site for a specific transcription factor, DSC1 (*DNA Synthesis Control*), which binds to MCB in a saturable and highly specific manner.

Analysis of the composition of DSC1 has led to an intriguing model for the mechanisms by which the requirements for DNA synthesis may be regulated and coordinated with other events of the cell cycle (Fig. 3). In cells carrying a null allele of *swi4*, DSC1 activity is normal. In contrast, deletion of *swi6* from the yeast genome impairs the cell cycle regulation of the DNA synthesis genes, implying that in addition to its role as a transcription factor in combination with Swi4, the *SWI6* gene product is also a component of DSC1 (Lowndes *et al.*, 1992a; Dirick *et al.*, 1992). A second component of DSC1 has been identified as a 120 kDa protein (p120), whose gene is as yet unknown (Dirick *et al.*, 1992). p120 can be cross-linked to MCB elements *in vivo*, unlike Swi6 which is not bound to DNA under similar conditions (Dirick *et al.*, 1992; N. Lowndes & L. Breedon, unpublished, cited in Merrill *et al.*, 1992). Analogous experiments have shown that Swi4 but not Swi6 binds to SCB (Primig *et al.*, 1992), suggesting that the *SWI6* gene product plays a regulatory role in the transcription factor activity of both SBF and DSC1. Moreover, it has been demonstrated that transcription by DSC1 can be induced by activating p34^{CDC28} even in the absence of protein synthesis, and the amount of Cln protein is rate limiting

Table 2. Genes whose expression is periodically regulated at the G1–S phase boundary.

Gene	Gene product
(a) Precursor biosynthesis	
<i>CDC8</i>	Thymidylate kinase
<i>CDC21</i>	Thymidylate synthase
<i>RNR1</i>	Ribonucleotide reductase subunit
(b) Replication machinery	
<i>RFA1</i>	Replication factor A subunit
<i>RFA2</i>	Replication factor A subunit
<i>RFA3</i>	Replication factor A subunit
<i>PRI1</i>	DNA primase I
<i>PRI2</i>	DNA primase II
<i>CDC9</i>	DNA ligase
<i>POL30</i>	PCNA
<i>POL1</i>	DNA polymerase I
<i>POL2</i>	DNA polymerase II
<i>DPB2</i>	DNA polymerase II subunit B
<i>DPB3</i>	DNA polymerase II subunit C
<i>POL3</i>	DNA polymerase III
(c) Coordination	
<i>CDC6</i>	Unknown
<i>DBF4</i>	Cdc7 kinase regulatory subunit

for this induction (Marini & Reed, 1992). It therefore seems likely that a component of DSC1 is a direct target for p34^{CDC28} protein kinase activity. Swi6 is a good candidate for this target since it is present in both SBF and DSC1, whose activities are both dependent on p34^{CDC28}.

A DSC1-like activity has been identified in extracts of the fission yeast, *Schiz. pombe* (Lowndes *et al.*, 1992b). Its components include the *cdc10* and *res1/sct1* gene products, which both show homology to Swi4 and Swi6 (Lowndes *et al.*, 1992b; Tanaka *et al.*, 1992; Caligiuri & Beach, 1993), both of which are essential for the G1-S phase transition. This DSC1-like complex has been shown to bind to the promoters of the essential and periodically transcribed genes *cdc22*, which encodes a subunit of ribonucleotide reductase, and *cdt1* (Lowndes *et al.*, 1992b; J. Hoffman & D. Beach, unpublished data cited in Caligiuri & Beach, 1993). Although only two genes have so far been shown to be regulated by this complex, the presence of this activity in *Schiz. pombe* has led to the proposal that it is a component of a conserved mechanism in eukaryotes for regulating periodic expression of genes involved in DNA synthesis (Merrill *et al.*, 1992). However, this has to be set against the observations that not all genes whose products are required for DNA synthesis are transcribed in this periodic manner. For example, three genes which, with *CDC21*, encode proteins that are required for dTTP synthesis do not contain the *MluI* motif (McIntosh *et al.*, 1986). Furthermore, genes encoding similar enzymic functions can exhibit different patterns of regulation in the two organisms. For example, DNA ligase is encoded by *CDC9* in *S. cerevisiae* and is regulated by DSC1, whereas in *Schiz. pombe* the equivalent protein is encoded by *cdc17+* which appears to be expressed independently of the DSC1-like activity (White *et al.*, 1986). Nevertheless, *CDC9* and *cdc17+* are functionally interchangeable and can complement appropriate mutants in the heterologous organism (Barker & Johnston, 1983). In addition, the regulation of the genes encoding DNA ligase and thymidylate synthase in *S. cerevisiae* can be divorced from the cell cycle by deletion of MCB with no apparent effect on growth or cell cycle timing (Johnston, 1990; Storms *et al.*, 1984). Therefore the coupling of a subset of genes involved in DNA metabolism to S phase thus appears not to be essential for cell cycle progression, but may rather ensure an optimal growth rate.

Initiation of DNA synthesis

Initiation of chromosomal DNA synthesis in eukaryotes is tightly coordinated in the cell cycle and several *cdc* mutants that block the initiation of chromosomal replication in *S. cerevisiae* have been characterized (see

Pringle & Hartwell, 1981, and Newlon, 1988, for reviews). Amongst these genes are three, *CDC4*, *DBF4* and *CDC7*, whose products function after p34^{CDC28} and which define a pathway of functional dependence leading to DNA synthesis, with the order of function within the pathway being *CDC28* → *CDC4* → *DBF4* → *CDC7* (Pringle & Hartwell, 1981; Johnston & Thomas, 1982; Newlon, 1988). This order, based on analysis of terminal morphology, execution points and the behaviour of temperature-sensitive mutants on release from arrest into media containing cycloheximide, defines the *CDC7* gene product as the last function operating in G1. However, there is as yet no direct evidence to support the view that the Cdc7 protein acts as a trigger of DNA synthesis and participates directly in chromosome replication.

The *CDC7* gene encodes a 58 kDa protein kinase (Patterson *et al.*, 1986; Bahman *et al.*, 1988; Hollingsworth & Sclafani, 1990). *CDC7* mRNA and protein levels are constant throughout the cell cycle, indicating that the protein kinase activity is regulated post-translationally (Sclafani *et al.*, 1988). One mechanism for this regulation was suggested initially from experiments in which it was demonstrated that some *cdc7* mutants can be suppressed by overexpression of *DBF4*, while *dbf4* mutants can be suppressed by elevated expression of *CDC7* (Kitada *et al.*, 1992). One interpretation of this is that the *CDC7* and *DBF4* gene products interact, as has now been demonstrated directly *in vivo* (Jackson *et al.*, 1993). The timing of this function for the Dbf4 protein is somewhat at odds though with the original genetic data that indicated that *CDC7* function was dependent on the prior completion of the step involving *DBF4* (see above). One possible explanation for this apparent discrepancy is that the Dbf4 protein is unstable as it contains a PEST-like sequence near its C-terminus (Kitada *et al.*, 1992; Jackson *et al.*, 1993). Thus, when *dbf4* cells are arrested, the Dbf4 protein is probably degraded so that on release from arrest, protein synthesis is required to resynthesize Dbf4 protein to allow cell cycle progression via activation of the Cdc7 protein kinase. This would therefore place *DBF4* and *CDC7* as interdependent functions in the pathway to S phase, a view that is supported by the identification of a mutation, *bob1-1*, which can suppress lethality in cells deleted for both *DBF4* and *CDC7* (Jackson *et al.*, 1993). By analogy with the activation of p34^{CDC28} protein kinase at START by interaction with Cln1, Cln2 or Cln3 proteins (Richardson *et al.*, 1989; Wittenberg *et al.*, 1990; Tyers *et al.*, 1992), the Dbf4 protein may act as a 'cyclin' to activate Cdc7 protein kinase activity at the G1-S phase boundary. This analogy can be extended since the precise timing of the *CDC7* activation after START may be determined in part by DSC1-dependent, periodic transcription of *DBF4*

(Chapman & Johnston, 1989; Kitada *et al.*, 1992). This model provides a sensitive, coordinately regulated pathway for coupling the completion of START to the synthesis of proteins needed for S phase and to the activation of DNA replication itself.

Summary

In *Saccharomyces cerevisiae*, START has been shown to comprise a series of tightly regulated reactions by which the cellular environment is assessed and under appropriate conditions, cells are committed to a further round of mitotic division. The key effector of START is the product of the *CDC28* gene and the mechanisms by which the protein kinase activity of this gene product is regulated at START are well characterized. This is in contrast to the events which follow $p34^{CDC28}$ activation and the way in which progress to S phase is achieved, which are less clear. We suggest two possible models to describe the regulation of these events.

Firstly, it is conceivable that the only post-START targets of the $p34^{CDC28}$ /G1 cyclin kinase complex are components of the SBF and DSC1 transcription factors. This would require that either SBF or DSC1 regulates *CDC4* function either directly by activating the transcription of *CDC4* itself or else indirectly by activating the transcription of a mediator of *CDC4* function in a manner analogous to the way in which the control of *CDC7* function may be mediated by transcriptional regulation of *DBF4* (Jackson *et al.*, 1993). Potential regulatory effectors of *CDC4* function include *SCM4*, which suppresses *cdc4* mutations in an allele-specific manner (Smith *et al.*, 1992) or its homologue *HFS1* (J. Hartley & J. Rosamond, unpublished). This possibility is supported by the finding that *CDC4* has no upstream SCB or MCB elements, whereas *SCM4* and *HFS1* have either an exact or close match to the SCB. This model would further require that genes needed for bud emergence and spindle pole body duplication are also subject to transcriptional regulation by DSC1 or SBF.

An alternative model is that the $p34^{CDC28}$ /G1 cyclin complexes have several targets post-START, one being DSC1 and the others being as yet unidentified components of the pathways leading to *CDC4* function, spindle pole body duplication and bud emergence. This model could account for the functional redundancy observed amongst the G1 cyclins with the various cyclins providing substrate specificity for the kinase complex. We suggest that a complex containing Cln3 protein is primarily responsible for, and acts most efficiently on, the targets containing Swi6 protein (SBF and DSC1), with complexes containing other G1 cyclins (Cln1 and/or Cln2 proteins) principally involved in activating the other pathways. However, there must be overlap in the

function of these complexes with each cyclin able to substitute for some or all of the functions when necessary, albeit with differing efficiencies.

This hypothesis is supported by several observations. Firstly, strains that are deleted for either *CLN1* or *CLN2* exhibit no observable phenotype, whereas strains that are deleted for both genes display an aberrant morphology that may be similar to that observed in cells defective in *CDC4* function (Hadwiger *et al.*, 1989). Secondly, deleting *CLN3* alone results in larger cells (Cross, 1988), which could indicate that the positive feedback loop at START B is impaired, and therefore only occurring slowly due to the use of less effective, overlapping Cln1/Cln2 functions. Perhaps most importantly though, it has recently been demonstrated that a burst of Cln3 activity is able to induce the transcription of both the SBF-regulated genes (*CLN1*, *CLN2*, *ORF* and *HCS26*) and the DSC1-regulated genes (*SWI4* and *CLB5*); furthermore, Cln3 is more potent at inducing this transcription than Cln1 (Tyers *et al.*, 1993). These data are consistent with our model outlined above. Further analysis of the function of the individual $p34^{CDC28}$ /G1 cyclin complexes will undoubtedly be informative with respect to both their roles in specific aspects of the G1–S phase transition as well as for the information that it will provide about this critical element of the cell division cycle.

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