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Control over the onset of DNA synthesis in fission yeast

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The fission yeast *Schizosaccharomyces pombe* has been used to identify gene functions required for the cell to become committed to the mitotic cell cycle and to initiate the processes leading to chromosome replication in S-phase.

Two gene functions *cdc2* and *cdc10* must be executed for the cell to traverse 'start' and proceed from G1 into S-phase. Before the completion of these two functions the cell is in an uncommitted state and can undergo alternative developmental fates such as conjugation. A third gene, *suc1*, has also been identified whose product may interact directly with that of *cdc2* at 'start'.

The molecular functions of the genes involved in the completion of 'start' have been investigated. The *cdc2* gene has been shown to be a protein kinase, suggesting that phosphorylation may be involved in the control over the transition from G1 into S-phase. The biochemical functions of the *cdc10* and *suc1* gene products have not yet been elucidated.

A control at 'start' has also been shown to exist in the budding yeast *Saccharomyces cerevisiae*. Traverse of 'start' requires the execution of the *CDC28* gene function. The *cdc2* and *CDC28* gene products (lower-case letters represent genes of *Schizosaccharomyces pombe*, and capital letters genes of *Saccharomyces cerevisiae*) are functionally homologous, suggesting that the processes involved in traverse of 'start' are highly conserved. An analogous control may also exist in the G1 period of mammalian cells, suggesting that the 'start' control step, after which cells become committed to the mitotic cell cycle, may have been conserved through evolution.

Introduction

During the cell cycle, the nucleus undergoes two dramatic events. The first is the replication of its chromosomes, which occurs during S-phase, and the second is mitosis, when the replicated chromosomes are segregated between the two daughter nuclei before cell division.

Yeasts provide us with an excellent model system for the study of these processes and their control, as they are very amenable to genetic and physiological analysis. We have studied the fission yeast, *Schizosaccharomyces pombe*, which has a typically eukaryotic cell cycle, with discrete G1-, S-, G2- and M-phases. In this paper we will review studies of the events that occur in late G1 as the cell first becomes committed to the mitotic cell cycle and then initiates the events that will lead to progress into S-phase.

Genetic and physiological analysis of G1- and S-phase

In rapidly growing cells the length of G1 is short, about 0.1 of a cell cycle (Nasmyth *et al.* 1979). At slow growth rates the length of G1 becomes greatly expanded and can occupy around 0.7 of the cell cycle (Nasmyth 1979). The absolute length of G2 + S-phase expands only slightly at slow growth rates. The duration of S-phase is essentially invariant, irrespective of the

duration of G1, but cannot be initiated until the cell has attained a critical mass (Nurse 1975; Nurse & Thuriaux 1977; Nasmyth *et al.* 1979).

A large number of conditional lethal mutants of *S. pombe* have been isolated that are blocked in cell-cycle progress (Nurse *et al.* 1976; Nasmyth & Nurse 1981; Yanagida *et al.* 1986). These define nearly 50 genes that are required for successful completion of the cell cycle. Physiological analysis of the mutants indicated that a number of them were defective in the processes leading up to, or required, during S-phase.

Mutants in the genes *cdc10*, *cdc20* and *cdc22*† fail to replicate their DNA upon shift to 36 °C, suggesting that the wild-type functions are required before DNA replication can occur. If these mutants are blocked in S-phase by using hydroxyurea at 25 °C, and then shifted to 36 °C and the hydroxyurea is removed, the cells undergo one round of DNA synthesis (Nasmyth & Nurse 1981). This suggests that these functions are completed before, or independently of, S-phase. Given the effect of these mutations upon DNA replication at 36 °C, it is likely that these gene functions must be completed for the cell to enter S-phase. Thus *cdc10*, *cdc20* and *cdc22* gene functions are classified as acting in G1 (Nasmyth & Nurse 1981). The *cdc17*, *cdc21*, *cdc23* and *cdc24* gene functions have been shown by similar experiments (Nasmyth & Nurse 1981) to be involved in S-phase. *cdc17* has been shown to encode DNA ligase (Nasmyth 1977) but no biochemical function has yet been ascribed to the *cdc10*, *cdc20*, *cdc21*, *cdc23* or *cdc24* genes.

'Start' control in fission yeast

The point at which yeast cells become committed to the mitotic cell cycle is known as 'start' and is the earliest gene controlled event of the mitotic cell cycle (reviewed by Nurse (1981)). Haploid cells that have traversed 'start' are committed to the mitotic cell cycle in the sense that they are unable to undergo alternative pathways such as conjugation. A large number of mutants blocked at different stages in the cell cycle were examined for their ability to conjugate when arrested (Nurse & Bissett 1981). Of those tested, *cdc10* mutants, which block cells in G1, were able to conjugate whereas *cdc22* mutants, which also block cells in G1, could not. The S-phase mutants *cdc17*, *cdc23* and *cdc24* and mutants in G2 and mitosis were also unable to conjugate. An exception to this was the mutant *cdc2*, previously characterized as defective in nuclear division (Nurse *et al.* 1976). It was found that a fraction of *cdc2* arrested cells were able to conjugate. Further characterization of this mutant demonstrated that the *cdc2* function was required twice in the mitotic cell cycle: first in G1, before S-phase, and then again in G2, before mitosis. Experiments with synchronous cultures of *cdc2* mutants indicated that only at the G1 block point were the cells able to conjugate (Nurse & Bissett 1981). These experiments demonstrate that the gene functions *cdc2* and *cdc10* must be executed before the cell can traverse 'start' and become committed to the mitotic cell cycle, and they also imply that G1 gene functions required for initiation of S-phase such as *cdc22* are only executed once the cell has passed 'start .

At rapid growth rates, completion of the *cdc10* function, followed shortly afterwards by S-phase, occurs early in the cell cycle. However, when the growth rate of the cell decreases, execution of the *cdc10* function, necessary for traverse of 'start', and therefore for commitment to the mitotic cell cycle, is delayed until later in the cell cycle, showing that under some

† Lower-case letters represent genes of *Schizosaccharomyces pombe* and capital letters genes of *Saccharomyces cerevisiae*.

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conditions traverse of 'start' becomes a major limiting step of the cell cycle (Nasmyth 1979).

'Start', representing the point of commitment to the mitotic cell cycle, is one of the two major control points in the *S. pombe* cell cycle. Its traverse requires firstly the cell to attain a critical mass, and secondly the execution of the *cdc2* and *cdc10* gene functions. A second control point acts in the G2 period and determines the timing of the initiation of mitosis. This mitotic control was revealed by the isolation of 'wee' mutants that undergo mitosis and cell division at about half the size of wild-type cells (Nurse 1975; Thuriaux *et al.* 1978). These 'wee' mutants are advanced through G2 into mitosis and thus define functions that are rate-limiting for the traverse of G2 and are involved in determining the timing of mitosis. The 'wee' mutants were shown to map to two genes, *wee1* and *cdc2* (Nurse & Thuriaux 1980). Genetic analysis suggested that *wee1* encodes an inhibitor of mitosis whereas the *cdc2* gene encodes a mitotic inducer. Mutations in the *cdc2* gene can then be of two sorts. One type are recessive, temperature sensitive lethal mutations causing cells to arrest either in G1 or in late G2 before mitosis, whereas the other type are dominant 'wee' mutations that cause cells to initiate mitosis prematurely. From these analyses it has become clear that one gene, *cdc2*, is involved at both major control points, acting first in G1 at the point of commitment to the mitotic cell cycle, and then again in G2, being involved in the mechanism that determines the timing of mitosis.

In this paper we will review the cloning and analysis of the two fission yeast genes known to be involved in the traverse of 'start', *cdc2* and *cdc10*, and also discuss the molecular analysis of *suc1*, an extragenic suppressor of some temperature sensitive mutants of the *cdc2* gene, which may also play a role in 'start'.

Cloning of the *cdc2*, *cdc10* and *suc1* genes

Plasmids containing the *cdc10* gene (Aves *et al.* 1985) have been isolated from a gene bank by their ability to permit the growth of a $cdc10^{ts}$ mutant strain at the restrictive temperature. The cloned DNA was shown to contain the *cdc10* gene by integrating plasmids via homologous recombination into the chromosome and then demonstrating that plasmid-linked markers were now closely linked to the chromosomal sites of the genes. A similar approach was also taken to clone the *cdc2* gene (Beach *et al.* 1982). In this case a second gene, in addition to *cdc2*, was isolated that permitted the growth of some $cdc2^{ts}$ mutants at the restrictive temperature when present on a multicopy plasmid. This gene was shown by genetic analysis to be unlinked to the *cdc2* locus and was named *suc1* (*su*ppressor of *c*dc) (Hayles *et al.* 1985). The gene isolated by this procedure was subsequently shown to be the same as a gene defined by chromosomal extragenic suppressors of $cdc2^{ts}$ mutations (Hayles *et al.* 1986).

Molecular characterization of the *cdc2*, *cdc10* and *suc1* genes

Northern blotting has demonstrated that the *cdc2* gene encodes a polyadenylated transcript of 1.6 kilobases (Durkacz *et al.* 1985, 1986). Given the central role of the *cdc2* gene function in cell-cycle progress we investigated whether changes in *cdc2* transcription were responsible for regulating entry and passage through the mitotic cell cycle. A culture of synchronous cells was prepared by selecting small cells at the beginning of the cell cycle by using an elutriator rotor, and RNA was prepared at various times. The level of the *cdc2* transcript was analysed by

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Northern blotting. Two mitotic cell cycles were sampled and no significant change in the steady-state level of the *cdc2* transcript was observed.

No change in the level of the *cdc2* transcript was found as cells underwent the transition from cell proliferation to stationary phase (Durkacz *et al.* 1986). A similar result was obtained for the *cdc10* gene, which encodes a 2.7 kilobase polyadenylated transcript (Aves *et al.* 1985). The steady-state level of the *cdc10* mRNA is unaltered during synchronous cultures or during the shift between rapid growth and stationary phase. The *suc1* gene encodes two transcripts of 0.8 and 1.2 kilobases. The steady-state level of these transcripts does not vary through the mitotic cell cycle (Hayles *et al.* 1986). Thus we may conclude that changes in the steady-state levels of the *cdc2*, *cdc10* and *suc1* gene transcripts do not play any role in regulating passage through, or exit from, the mitotic cell cycle.

The *cdc2*, *cdc10* and *suc 1* genes have all been sequenced (Hindley & Phear 1984; Aves *et al.* 1985; J. Hindley & G. A. Phear, unpublished data). The *cdc2* gene has the potential to encode a 34 kDa protein after removal of four intervening sequences. The *cdc10* gene contains no intervening sequences and could potentially encode a protein of 85 kDa. The *suc1* gene sequence reveals no long open reading frame. The gene contains two putative intervening sequences suggested by the presence of consensus splice donor and acceptor sites in the DNA sequence. Their existence has not yet been confirmed, however, by mapping of the transcripts. The relation between the two transcripts is unknown though evidence suggests that they are overlapping, transcribed from the same strand and share a common 5′ end (Hayles *et al.* 1986; S. J. Aves, unpublished data). Removal of both introns would result in a protein product of 10 kDa.

Computer searches have failed to identify any proteins with significant homologies to the predicted *cdc10* and *suc1* proteins. To try to gain further insights into their biochemical functions we are currently raising antibodies to the *cdc10* and *suc1* proteins. The computer search did reveal a number of significant homologies with the *cdc2* protein. At the amino acid level, about 20% homology is seen with the catalytic subunit of cAMP-dependent protein kinase and a number of putative protein kinases from the *src* family of oncogenes. A much higher level of homology, about 62%, was found with the cell division cycle gene *CDC28* of the budding yeast *Saccharomyces cerevisiae* (Hindley & Phear 1984; Lorincz & Reed 1984; Nurse 1985).

Comparison of the regions conserved between *cdc2* and *CDC28* allows the significance of the approximately 20% homology observed between *cdc2* and protein kinases to be assessed. This analysis shows only 8% identity between the sequences of *cdc2*, *CDC28*, v-*mos* and the catalytic subunit of cAMP-dependent protein kinase, at first sight, not very significant. However, most of this similarity may be accounted for by the presence of two functionally important regions within the proteins; an ATP binding site of the sort found in protein kinases and a phosphorylation site (Nurse 1985).

To maximise the homology between the *cdc2* and *CDC28* gene reading frames, it was necessary to propose the existence of four intervening sequences in the *cdc2* gene. The *CDC28* gene has no intervening sequences (Lorincz & Reed 1984). The presence of these four introns was subsequently confirmed by S1 mapping (Durkacz *et al.* 1986). The *CDC28* gene, like the *cdc2* gene, has two points of action during the mitotic cell cycle, being required in G1 at start, and again later in the cell cycle to initiate mitosis (Piggott *et al.* 1982). The *cdc2* and *CDC28* genes are closely related with respect to their molecular function. A *CDC28* gene introduced

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by transformation into an *S. pombe* $cdc2^{ts}$ mutant will rescue the mutant function and permit the cells to complete the cell cycle at the restrictive temperature (Beach *et al.* 1982). The *cdc2* gene, after removal of the four intervening sequences, will also complement *CDC28* temperature sensitive mutants (Booher & Beach 1986). This demonstrates the near functional equivalence of these two gene products.

Analysis of the *cdc2* gene product

The similarities between *cdc2* and certain protein kinases strongly suggest that the *cdc2* gene product is a protein kinase and probably also a phosphoprotein. To confirm this and to investigate *cdc2* function we have prepared *cdc2* specific antibodies. After removal of the four intervening sequences, the *cdc2* gene has a single, long, open, reading frame 297 amino acids in length (Hindley & Phear 1984). Three peptides were synthesized from the predicted amino acid sequence of the protein, and after coupling to a carrier protein they were used to immunize rabbits. The resulting heteroantisera detect an *S. pombe* protein of 34 kDa. The level of this protein is greatly increased by fusion of the *cdc2* gene to a strong promoter, showing that is is *cdc2* protein specific (Simanis & Nurse 1986). Immunoprecipitation from cell extracts labelled with ^{32}P has demonstrated that the *cdc2* protein is phosphorylated *in vivo*. Immune complex kinase assays were then done, and established that the immunoprecipitates which contain *cdc2* protein possess protein kinase activity, which is thermolabile when prepared from $cdc2^{ts}$ mutant strains. This strongly indicates that the *cdc2* protein is indeed a protein kinase. The possibility that the *cdc2* protein is tightly associated with a protein kinase rather than the possibility that it possesses intrinsic protein kinase activity cannot, however, be formally eliminated with the available data.

No significant changes in gross phosphorylation state or protein level of *cdc2* were observed in synchronous cultures of wild-type cells (Simanis & Nurse 1986). However, in exponentially growing wild-type cells, the G1 period is very brief. This is because at division the cells are already big enough to pass start and so enter S-phase after a minimum time in G1 (Nurse & Thuriaux 1977). To investigate whether any changes in *cdc2* protein level or phosphorylation state occur during passage of start in G1, we analysed these parameters during exit from the mitotic cell cycle. Cells in exponential growth were starved of nitrogen, which causes them to complete the mitotic cell cycle in progress and accumulate in G1 before start. No significant changes in protein level were observed as the cells arrested in G1. However, it was found that within 3 h after the shift to nitrogen-free medium, when most cells had accumulated in G1, the *cdc2* protein had become dephosphorylated. This was paralleled by a decrease of *in vitro cdc2* protein kinase activity, which had fallen to less than 5% of its initial value by 3 h, suggesting that dephosphorylation of the *cdc2* protein may lead to a decrease in its protein kinase activity. When starved cells are shifted back into complete medium the protein kinase activity is restored after a 3 h lag period, about the time when the cells would be expected to be traversing start. Cell division follows 2–3 h later, approximately equivalent to a period of one cell cycle (Simanis & Nurse 1986).

Taken together, these findings are consistent with the idea that while the *cdc2* protein remains phosphorylated, the protein kinase activity is maintained and causes cells to remain committed to the mitotic cell cycle. Upon nutrient deprivation the *cdc2* protein becomes dephosphorylated and loses its protein kinase activity, and as a consequence cells are unable

to initiate the next cell cycle and accumulate in G1. It is therefore possible that modulation of the *cdc2* protein kinase activity perhaps by changes in the phosphorylation state of the protein may be part of the mechanism by which the cell controls the transition from rapid growth to stationary phase. However, our current data do not permit us to exclude other possibilities, for instance that some modification of the *cdc2* protein other than dephosphorylation occurs to effect the decrease in protein kinase activity upon nutrient starvation.

The *suc1* gene function probably interacts with the *cdc2* protein

The *suc1* mutants, or overexpression of the wild-type *suc1* gene (*suc1* mutants), could suppress $cdc2^{ts}$ mutations by opening up a bypass pathway that circumvents the requirement for the *cdc2* gene function. If this were so, we would expect the various *suc1* mutants to allow all $cdc2^{ts}$ mutants to grow at the restrictive temperature. It was found, however, that two of the $cdc2^{ts}$ alleles tested, *cdc2*-M35 and *cdc2*-M63, could not be suppressed by *suc1* mutation or *suc1* overexpression. It was also demonstrated that good suppression of $cdc2^{ts}$ alleles is only achieved at 32 °C, suggesting that some residual *cdc2* activity is required to permit a cell to divide at the restrictive temperature in the presence of mutant *suc1* or *suc1* overexpression (Hayles *et al.* 1986). The allele-specific nature of the suppression therefore suggests that *suc1* function interacts in some way with that of *cdc2*, rather than by opening up a bypass pathway.

One way the two gene functions could interact with each other is for *suc1* mutants to increase the $cdc2^{ts}$ transcript level. It has already been demonstrated that overproduction of the transcript in some $cdc2^{ts}$ alleles can restore wild-type activity (Durkacz *et al.* 1985). However, examination of the *cdc2* transcript level in *suc1* mutants showed no gross changes in level compared with wild-type grown at similar temperatures. Thus the suppressing effects of the *suc1* mutants do not arise through changes in $cdc2^{ts}$ gene transcription (Hayles *et al.* 1986). This suggests that interaction of the two gene functions occurs at a post-transcriptional level and that the *cdc2* and *suc1* protein molecules may interact with each other.

Further evidence that the *cdc2* and *suc1* protein molecules interact has been obtained by overexpression of *suc1* (Hayles *et al.* 1986). Fusion of the wild-type *suc1* gene to the strong constitutive *S. pombe* alcohol dehydrogenase promoter results in cells that are delayed in entering mitosis until they are approximately twice the normal size. Effects are also seen in meiosis where the second meiotic nuclear division is blocked. Similar phenotypes are also observed in cells which have partial *cdc2* protein activity (Hayles *et al.* 1986). It is therefore possible that if the *suc1* and *cdc2* proteins interact, the complex in the *suc1* overexpressing strain might be in some way aberrant, perhaps leading to less efficient *cdc2* gene function, thereby causing the observed effects on mitosis and meiosis.

The *suc1* gene is essential for cell viability

The *suc1* gene is also essential for cell viability (Hayles *et al.* 1986). This was shown by replacing part of the putative open reading frame with the *ura4* gene. These strains were then sporulated and tetrad analysis was done. In all tetrads a 2:2 segregation of growth to non-growth was observed. Only $ura4^{-}$ spores could germinate, demonstrating that strains containing the *suc1* gene disrupted by *ura4* were inviable and the spores fail to form colonies (Hayles *et al.* 1986). In many cases the spores germinated and underwent two or three divisions

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before cell death. The terminal phenotype of the cells was not uniform. Many cells became elongated and swollen although the degree of elongation was variable. About 30% of the cells failed to elongate though they were swollen and misshapen. Both phenotypes were usually observed within a single microcolony. A similar range of phenotypes was observed after loss of an unstable plasmid from a strain containing the *suc1* deletion.

These experiments show that the *suc1* gene encodes a product that is essential for cell growth. Absence of the gene product results in a heterogeneous terminal phenotype, which indicates that in some cells cell division is impaired but growth continues whereas in others little growth can take place. These variable phenotypes suggest that the *suc1* gene product plays several roles in the cell, one concerned with progress though the cell cycle, involving interaction with the *cdc2* protein, and the second concerned with cellular growth involving interaction with other, as yet unidentified protein molecules. An attractive proposition is that the *suc1* gene product interacts with and facilitates the action of a number of protein kinases among which is that encoded by *cdc2*. Of these, some could be required for cell cycle progress whereas others might be involved in cellular growth.

Events in G1 after 'start'

We will now consider what is known of the events that occur once the cell has become committed to the mitotic cell cycle and set in motion the processes leading to S-phase.

Two genes, *cdc20* and *cdc22*, are known to act in late G1 after 'start'. The *cdc22* gene has been cloned (Gordon & Fantes 1986). It has been shown that the *cdc22* steady-state transcript level is periodic through the cell cycle, reaching a peak during late G1–S phase. The *cdc20* gene has not yet been cloned.

It has also been demonstrated that histone H2A and H2B steady-state transcript levels vary periodically throughout the cell cycle, peaking in S-phase (Aves *et al.* 1985). The pattern of H2B transcript accumulation has been monitored in a variety of *cdc* mutants that block at different times during G1 and S phase (Matsumoto *et al.* 1987). Cells blocked before 'start' with *cdc10* fail to accumulate H2B transcripts above basal levels, whereas in cells blocked after 'start' with *cdc22* H2B transcripts do accumulate. Transcript levels are also found to increase in another G1 mutant *cdc20*. These experiments establish that the signal for increase of histone transcript level in preparation for S-phase is given in late G1 before any DNA synthesis occurs. In the *cdc20* and *cdc22* mutants the transcript levels only fall slowly, which suggests that the signals that lead to the decrease in level are not given correctly in G1 arrested cells.

Little is known of the molecular function of the genes *cdc21, cdc23* and *cdc24* as they have not yet been cloned. The *cdc17* gene has been shown to encode DNA ligase (Nasmyth 1977), and has been cloned (Johnston *et al.* 1986). The *cdc17* gene transcript steady-state level does not vary significantly through the mitotic cell cycle (White *et al.* 1986). It is interesting to contrast this with the situation that applies in the homologous gene *CDC9* in *Saccharomyces cerevisiae*. This gene also encodes DNA ligase, and is transcribed periodically during the mitotic cell cycle, peaking at the G1–S boundary, before histone transcription. The gene will however complement *cdc17* mutations of *S. pombe*.

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Summary of 'start' control in *S. pombe*

We have investigated the functions that are involved in the progress through G1 into S-phase in fission yeast. Traverse of 'start', after which the cell is committed to the mitotic cell cycle, requires execution of the *cdc2* and *cdc10* functions. The biochemical activity of the *cdc10* protein is unclear, but the *cdc2* protein has been demonstrated to be a protein kinase. This strongly suggests that phosphorylation of a specific set of proteins is required to pass 'start'. The *cdc2* protein kinase becomes activated at 'start' and may be regulated by phosphorylation. The *suc1* gene encodes a protein that probably interacts with the *cdc2* protein at 'start'.

Once 'start' has been passed, the cell cannot undergo alternative fates such as conjugation and initiation which are the events that will lead to S-phase. These include the execution of the *cdc20* and *cdc22* functions and the accumulation of histone transcripts in the preparation for initiation of chromosome replication during S-phase.

'Start' control in other eukaryotes

An analogous 'start' control has been identified in the budding yeast *Saccharomyces cerevisiae* (Hartwell 1974). As in *S. pombe*, 'start' in *S. cerevisiae* is both a point of commitment to, and a rate limiting step for entry into, S-phase. Before budding yeast cells have completed 'start' they are in an uncommitted state and can undergo the alternative developmental pathway of conjugation if challenged to do so. Budding yeast cells must also attain a critical cell size before they can complete the 'start' control step (Hartwell & Unger 1977). Under poor nutrient conditions, cells fail to grow sufficiently and become arrested in G1 before 'start'.

The molecular mechanism acting at 'start' in *S. cerevisiae* also appears to have been conserved. Four 'start' genes have been identified: *CDC28, 36, 37, 39* (Reed 1980). As described earlier, *CDC28* is homologous to the *cdc2* gene of *S. pombe*. Both genes encode a protein kinase that is itself phosphorylated (Simanis & Nurse 1986; Reed *et al.* 1985) and the *CDC28* gene product can rescue mutants of *cdc2* including a deletion of the *cdc2* gene (Beach *et al.* 1982; T. Carr, personal communication). This suggests that both gene products have similar functions although the two organisms are considerably diverged in evolutionary terms (Husymans *et al.* 1983).

In mammalian cells, under a variety of suboptimal growth conditions, cells become arrested in G1. Pardee (1974) has shown that cells arrest at the same point in G1, which has been termed the R (Restriction) point. The R point appears to be a time at which the cell becomes independent of growth factors present in serum and can proceed towards S-phase in the absence of these factors. Cells become serum independent about 2 h before the onset of DNA synthesis (Yen & Pardee 1978). A similar step has been described by Zetterberg & Larsson (1985). They have shown that a control step about 3–4 h after mitosis is a point at which cells become independent of growth factors. Cells that are 3–4 h post-mitosis, if deprived of serum, become arrested in G1 whereas cells later in G1 are unaffected and can proceed towards DNA synthesis. The difference in the timing of this step and the R point probably reflects the different cell types used.

The R point has some similarities with 'start' in the two yeasts in that it is a point at which the cell becomes committed to undergoing DNA synthesis. There is also evidence that in certain *in vitro* differentiation systems mammalian cells, like yeast cells, may differentiate from

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an uncommitted prereplicative phase before the R point. Scott *et al.* (1982) using an adipocyte differentiation system have shown that cells apparently cannot undergo differentiation once they have completed a step in late G1. These cells could only differentiate from a distinct point in G1, termed GD.

In the two yeasts the passage of 'start' is strongly correlated with the attainment of a critical cell size (Nurse & Thuriaux 1977; Nasmyth 1979; Hartwell & Unger 1977). However, a clear relation between cell size and cell-cycle progress has not been established in mammalian cells, although there is some indication that a size requirement can act over the onset of DNA synthesis in some cells (Shields *et al.* 1978; Killander & Zetterberg 1965).

To establish if a 'start' control does exist in mammalian cells, it will be important to determine if these cells contain homologues to the 'start' genes such as *cdc2* and *cdc10*. We are currently investigating this possibility.

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