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## THE CENTRAL ROLE OF A CDK IN CONTROLLING THE FISSION YEAST CELL CYCLE

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Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path; nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual law of Nature by careful investigation of rarer forms of disease. For it has been found in almost all things, that what they contain of useful or applicable nature is hardly perceived unless we are deprived of them, or they become deranged in some way. (*From a letter written six weeks before William Harvey's death in 1657.*)

### I. INTRODUCTION

The cell cycle is the process by which cells reproduce themselves, and controls acting over the cell cycle ensure an orderly progression through this reproductive process. An important element controlling cell cycle progression in eukaryotes is the cyclin dependent kinase (CDK) family of protein kinases, which regulate passage through the major events of the cell cycle in all eukaryotes. In this paper attention is focused on the simple unicellular eukaryote fission yeast *Schizosaccharomyces pombe*. Emphasis is placed on the role of *cdc2p*, the catalytic core of the CDK protein kinase that controls the cell cycle in fission yeast. Cell cycle controls are more elaborate in multicellular eukaryotes, but focusing on a single model system has the advantage of providing a more coherent and complete description of how the controls operate than is yet possible in more complex systems. Also, understanding generated with a simple model such as fission yeast can be of use in unravelling the more elaborate and redundant controls operative in more complicated Metazoan cells.

## II. CELL CYCLE CONTROLS

The cell cycle is often defined as the period in the life of a growing cell between the formation of the cell by the division of its mother and the time when the cell itself divides to form two daughters. This is a useful definition because it places emphasis on the fact that the cell cycle usually occurs only when cells are already in an active state of growth, and thus discussion about cell cycle control is not confused with cell growth control. The latter regulates the transition from a non-growing to a growing state. In a free-living unicellular organism such as fission yeast, growth controls are concerned with the availability of essential nutrients in the growth medium, while in a mammalian cell growth, controls are concerned with growth factor signals that reflect the social interactions of cells operative in a multicellular organism. These growth controls are of great importance but will not be dealt with here. Consideration will be confined to the controls regulating progression through the cell cycle of a cell that is already actively growing.

For the cell cycle to be successful it is necessary that at cell division the two daughters each receive a full complement of all the components necessary for cell survival. Of particular importance is the genome, because each new daughter cell should contain a complete set of genes. Two events present in all normal eukaryotic cell cycles are essential to achieve this end. These are S-phase (S), when the DNA making up the genome is replicated, and M-phase (M) or mitosis in the mitotic cell cycle, when the replicated chromosomes are segregated into the two daughter cells. An important aspect of cell cycle control is concerned with regulating the onset of these two events such that they occur in the correct sequence of S-phase followed by mitosis, and only once in each cell cycle. Therefore much of the work considered here is concerned with elucidating the mechanisms that control onset of these two events.

Precisely replicating and accurately segregating the genome is a complex process that can go wrong. Cell cycle checkpoint controls ensure that a cell does not divide with a partly or incorrectly replicated genome and that mistakes do not occur during chromosomal segregation. If a mistake occurs during S-phase that blocks DNA replication, then the onset of mitosis is also blocked. Similarly, if chromosome segregation is defective, then cell division is blocked. These blocks give time for the mistakes and defects to be corrected, after which the blocks are relieved and normal cell cycle progression is allowed. Checkpoint controls are crucial for the fidelity of the genomic reproductive process and therefore also form an important part of cell

cycle control. Also of relevance in this regard is the mechanism that ensures that there is only one S-phase per cell cycle. Failure in this mechanism would lead to an increase in cell ploidy, and although each daughter would still receive a full complement of the genes, the ploidy changes would be detrimental for both proper development and sexual reproduction. These controls, which are closely related to those regulating the onset of the events of S-phase and mitosis, maintain genomic stability, which is essential for the survival of both unicellular and multicellular organisms.

Elucidation of these controls is difficult because of their complexity, but is much assisted by a genetic approach. Specific mutants disrupt single elements acting in the controls, and careful study of the subsequent mutant phenotype or pathology is revealing about the normal processes involved. When this is combined with molecular genetics allowing the cloning of the relevant genes, it is then possible to work out the molecular mechanisms underlying the controls. Fission yeast is a genetically amenable organism that is particularly suited for such a genetic analysis of cell cycle controls (Nurse, 1975; Nurse, et al., 1976). Work with this organism has been illuminating about most of the controls outlined above. In particular it has drawn attention to the *cdc2* gene (encoding *cdc2p*) as an important element in the controls (Nurse, 1990), and the mode of action and regulation of *cdc2p* will now be summarized.

### III. ACTION AND REGULATION OF *cdc2p*

As mentioned above, *cdc2p* is a catalytic protein kinase core (Simanis and Nurse, 1986). It is 34 kD in size and must be complexed with a cyclin regulatory subunit to become active. There are four cyclins in fission yeast; three of these are B-cyclins, *cdc13p* (Hagan et al., 1988), *cig1p*, and *cig2p*, all of which have roles in the cell cycle. The fourth is *puc1p*, which is closely related to the CLN cyclins of budding yeast (Forsburg and Nurse, 1991). In fission yeast *puc1p* has no clearly identified role in the cell cycle, but does have a minor role in controlling conjugation and meiosis (Forsburg and Nurse, 1994). As well as this requirement for cyclin binding, *cdc2p* needs to be phosphorylated on threonine T167 for active protein kinase activity (Gould et al., 1991). This T167 phosphorylation may stabilize cyclin binding.

The *cdc2p* protein kinase activity is subject to three further types of regulation. The first is an inhibitory phosphorylation of tyrosine Y15 located in the active site of the enzyme (Gould and Nurse, 1989). When phosphory-

lated on Y15 the protein kinase activity is reduced although not eliminated. Specific protein kinases (primarily *wee1p*) (Russell and Nurse, 1987) and protein phosphatases (primarily *cdc25p*) (Russell and Nurse, 1986; Gould et al., 1990) regulate the phosphorylation state of Y15. The second type of regulation is association of the *cdc2p* CDK with a specific CDK inhibitor *rum1p* (Moreno and Nurse, 1994; Correa-Bordes and Nurse, 1995). This association completely inhibits the *cdc2p-cdc13p* CDK protein kinase activity at least *in vitro*, but is less effective against the *cdc2p-cig1p* and *cdc2p-cig2p* CDKs. The third type of regulation is controlled proteolysis of the cyclin component of the *cdc2p* CDK. Proteolysis of *cdc13p* is brought about by the proteasome, although *rum1p* may play a role in efficiently directing *cdc13p* to the proteasome. All three types of regulation are used at different stages of cell cycle regulation in fission yeast.

#### IV. CONTROLLING MITOTIC ONSET

The best understood cell cycle transition in fission yeast is the control acting at mitotic onset. This is brought about by the *cdc2p-cdc13p* CDK, although *cdc2-cig1p* CDK may play a very minor role. The complex of *cdc2p* with *cdc13p* begins to form around S-phase, and sufficient complex for adequate mitotic protein kinase activity is present by the first part of G2 (Moreno et al., 1989). The main control determining the timing of mitotic onset involves Y15 phosphorylation (Gould and Nurse, 1989). As soon as the *cdc2p-cdc13p* complex is formed, Y15 becomes phosphorylated primarily by the *wee1p* protein kinase, although the *mik1p* protein kinase also plays a contributory role. This leads to a low level of *cdc2p-cdc13p* protein kinase activity from S-phase to G2 during the cell cycle. The Y15 phosphate is removed primarily by the *cdc25p* protein phosphatase, although the *pyp3p* protein phosphatase also plays a contributory role. When Y15 is dephosphorylated, a high level of *cdc2p-cdc13p* protein kinase is formed, which brings about mitosis. Thus activation of the *cdc2p-cdc13p* CDK at the end of G2 depends mainly on the balance between the activities of the *wee1p* protein kinase and the *cdc25p* protein phosphatase. Activation occurs when the cell attains a critical mass (Nurse, 1975), and removing *wee1p* (Russell and Nurse, 1987) or over-expressing *cdc25p* (Russell and Nurse, 1986) advances cells into mitosis at a reduced cell mass.

When the *cdc2p-cdc13p* CDK is fully activated, mitosis takes place. A number of key substrates required for the major events of mitosis are thought

to become phosphorylated at this time (Moreno and Nurse, 1990). These include formation of a mitotic spindle, chromosome condensation, and changes in the nuclear envelope that occurs as the nucleus extends during mitosis. Little work has been done on these substrates, but *cdc2p* has been found in the spindle pole body (of obvious relevance for forming the mitotic spindle), and when a vertebrate lamin (a component of the nuclear envelope) is expressed in fission yeast, it is phosphorylated by *cdc2p-cdc13p* and becomes dispersed during mitosis (Enoch et al., 1991). To get out of mitosis, the *cdc2p-cdc13p* CDK activity must be much reduced. This is brought about by specific *cdc13p* proteolysis and requires the action of *nuc2p* and *cut9p*, two components of the proteasome. When these components are defective, cells block in mitosis because of their failure to degrade *cdc13p*, and as a consequence a high level of *cdc2p-cdc13p* protein kinase activity is maintained, which blocks mitotic exit. Only when *cdc2p-cdc13p* activity is at a low level can cells exit mitosis and complete the cell cycle.

#### V. CONTROLLING S-PHASE ONSET

Onset of S-phase is less well understood. The primary cyclin involved is *cig2p*, and *cdc2p-cig2p* CDK protein kinase activity rises to a peak at the G1/S boundary. If *cdc2p* is inactive, then cells fail to enter S-phase, but the mechanisms controlling activation and inactivation of *cdc2p-cig2p* have yet to be elucidated.

*cig2p* is not the only B-cyclin that can act at the G1/S boundary. Both *cig1p* and *cdc13p* can substitute for *cig2p* if the latter cyclin is not present. Usually *cdc2p-cig2p* is activated earlier in the cell cycle, and so neither *cig1p* nor *cdc13p* normally have any function at G1/S. However, if the *cig2* gene is deleted, then either *cig1p* or *cdc13p* can bring about S-phase (Fisher and Nurse, 1996). When both *cig1* and *cig2* are deleted, the mitotic-cyclin *cdc13p* becomes essential for onset of both S-phase and mitosis. Activity associated with *cdc2p-cdc13p* increases during the cell cycle, first bringing about S-phase and then mitosis because the latter event requires a greater level of activity.

The molecular mechanism by which *cdc2p* brings about S-phase is likely to involve *cdc18p*. This protein plays a key role for the initiation of DNA replication in fission yeast (Kelly et al., 1993; Nishitani and Nurse, 1995). When *cdc18* is deleted, onset of S-phase is blocked, and when *cdc18* is over-expressed, multiple rounds of DNA replication occur even in the ab-

sence of continued protein synthesis. This identifies *cdc18p* as a major rate-limiting step for the initiation of DNA replication. *cdc18p* complexes with *orp1p*, an *orc1p*-related protein thought to be part of an origin recognition complex (ORC) associated with origins of replication, and with *cdc21p*, a *mcm4p*-related protein (Grallert and Nurse, 1996). At present, speculation on the modes of *cdc18p* action must be tentative, but it could be imagined that *cdc18p* is required for S-phase onset at a point downstream of *cdc18p* accumulation, and so *cdc2p*-*cig2p* CDK protein kinase activity may act at the point where *cdc18p* activates ORCs.

## VI. BLOCKING M UNTIL S IS COMPLETE

An important cell cycle checkpoint control is the block over mitosis that occurs when S-phase is incomplete (Enoch and Nurse, 1991). This control is revealed when DNA replication is blocked with the inhibitor hydroxyurea, which blocks mitotic onset (Enoch and Nurse, 1990; Enoch et al., 1992). The control works through *cdc2p* because mutants that reduce *cdc2p* Y15 phosphorylation fail to block mitosis even though S-phase is incomplete. Various genes have been identified that are required for this checkpoint blocking signal. Mutants in these genes lead to *hydroxyurea* sensitivity and so the genes implicated are called *hus* genes. Many *hus* genes are also radiation-sensitive and are found to be identical to previously identified *rad* genes. This has led to the view that there is at least some overlap between the mechanisms blocking mitosis due to failures to complete DNA replication and due to DNA damage. The present view is that blocks in DNA replication and damage to DNA send signals that are communicated to the *cdc2p*-*cdc13p* mitotic CDK by *hus/rad* gene pathway. Although both checkpoints share some common features, other gene functions may be unique to each pathway.

*cdc18p*, together with a specific subset of proteins required for the initiation of DNA replication, is crucial for sending the signal that S-phase is incomplete. When *cdc18* is deleted, cells fail to initiate S-phase and also fail to block the subsequent mitosis. This suggests that *cdc18p* is required both to initiate DNA replication and also for sending the signal that DNA replication is in process. Other replication proteins that behave in a similar way include *orp1p* and DNA polymerase alpha (D'Urso et al., 1995). All three proteins are required at an early stage in the initiation of DNA replication. Loss of other replication proteins acting later in the initiation process, including DNA polymerase delta and epsilon and PCNA, block S-phase and

also block mitotic onset. This indicates that at these later mutant blocks the checkpoint signal monitoring that DNA replication is in process has been sent. This can be understood if the checkpoint signal is sent by the formation of replicative complexes acting at the initiation of DNA replication. These are formed at the onset of S-phase, and their presence signals that DNA replication is in process. All these replication complexes are consumed during the process of DNA replication, and so at the end of S-phase no further inhibitory signal is sent and cells can undergo mitosis. Deleting genes that block late in the replication process (e.g., DNA polymerase delta) does not affect formation of the replicative complexes, and so mitosis is blocked. In contrast, deleting genes that block early (e.g., *cdc18p*) prevents the formation of the complexes, and so no inhibitory signal is sent and cells enter mitosis.

An early G1 cell has also not completed S-phase and should not undergo mitosis, and yet it has not yet formed any replicative complexes and so cannot block onset of mitosis by the mechanism described above. During this phase of the fission yeast cell cycle it is *rum1p* that prevents mitotic onset (Moreno and Nurse, 1994; Correa-Bordes and Nurse, 1995). When a cell completes mitosis and enters G1, *rum1p* levels rise, inhibiting any *cdc2p-cdc13p* CDK present and promoting *cdc13p* protein turnover. This mechanism ensures that early G1 cells do not enter mitosis. However, if *rum1* is deleted, cells blocked in early G1 will proceed to mitosis even though S-phase has not taken place. *rum1*-deleted cells blocked in S-phase using hydroxyurea do not proceed to mitosis because the Y15 phosphorylation checkpoint control is still intact in these cells.

## VII. BLOCKING S UNTIL M IS COMPLETE

When a fission yeast cell is arrested in G2, it does not re-initiate another S-phase. In other words, S-phase can only take place when the mitosis of the previous cell cycle is completed, that is, there can only be one S-phase per cell cycle. This checkpoint control also involves the *cdc2p-cdc13p* CDK that inhibits re-initiation of DNA replication during the G2 phase of the cell cycle. When *cdc13* is deleted (Hayles et al., 1994) or the specific *cdc2p-cdc13p* CDK inhibitor *rum1p* is over-expressed (Moreno and Nurse, 1994), there is a very low level of *cdc2p-cdc13p* CDK protein kinase activity present in the cell. As a consequence, G2-arrested cells re-initiate DNA replication and so cell ploidy increases.

The advantage of this inhibitory control mechanism is that it explains why there is only one S-phase during each cell cycle. In the normal cell cycle, *cdc2p-cdc13p* protein kinase activity has to be reduced to a very low level before a cell can exit mitosis. Such reduction would not only lead to mitotic exit but also to release from the block acting over the re-initiation of DNA replication. Thus the *cdc2p-cdc13p* mitotic CDK must have two roles during G2. It prepares the cell for mitosis, the legitimate event for a G2 cell, and it prevents the cell from undergoing S-phase, the illegitimate event for a G2 cell. Upon exit from mitosis and entering G1, the second inhibitory function is lost because S-phase is now the legitimate event for a G1 cell.

The manner by which the *cdc2p-cdc13p* CDK inhibits re-initiation of DNA replication is not known, but it is of interest that *cdc18p* has *cdc2p* consensus phosphorylation sites that appear to be inhibitory for *cdc18p* function. Perhaps phosphorylation of *cdc18p* and of other proteins may prevent replicative complexes from being formed. This can be incorporated into a two-step mechanism for the initiation of DNA replication (Stern and Nurse, 1996). In the first step, *cdc2p* CDK protein kinase activity must be reduced to a level that allows formation of the initiating replicative complexes on origin regions of the DNA. In the second step, *cdc2p* CDK (usually complexed with *cig2p*) protein kinase activity must rise to a level that brings about initiation. In such a scheme the second step automatically inhibits the first step. This would prevent the re-establishment of replicative complexes on regions of the DNA that have already been replicated, thus ensuring that no region is replicated twice during one S-phase.

### VIII. EVOLUTIONARY ASPECTS

From the above account it can be seen that the *cdc2p* CDK plays a key role in maintaining an orderly progression through the cell cycle. In early G1, *cdc2p* CDK activity is very low, allowing step one for the initiation of DNA replication. A rise of *cdc2p-cig2p* CDK activity at the end of G1 carries out step two and brings about S-phase, while at the same time it prevents the re-replication of any region of DNA that has already been replicated. The continued presence of *cdc2p* CDK activity in the form of *cdc2p-cdc13p* during G2 prevents another S-phase from taking place. Finally, further activation to a higher level of *cdc2p-cdc13p* leads to mitotic onset. To exit mitosis and enter G1 of the next cell cycle, *cdc2p* CDK activity must fall to a

very low level again. Therefore, orderly progression through the cell cycle is driven by increasing activity associated with the *cdc2p* protein kinase.

Although in fission yeast these transitions are normally brought about by different cyclin complexes, primarily *cdc2p-cig2p* and *cdc2p-cdc13p*, it is possible for cell cycle progression to be regulated entirely by the single CDK *cdc2p-cdc13p*. This may echo the situation present in more primitive ancient eukaryotes when the cell cycle may have been controlled by a single CDK, the activity of which gradually increased throughout the cell cycle, bringing about the major cell cycle transitions and establishing the major cell cycle checkpoints.

What is not clear, however, is why the eukaryotic cell should have invested so much control in a single CDK that regulates the onset of such different events as S-phase and mitosis. It is possible that in the primitive ancient eukaryotic cell, DNA replication and chromosome segregation were not distinct events (Nurse, 1994). Bi-directional replication results in two replicating forks moving away from each other. This separating process may have also been the primitive mechanism for chromosomal segregation if there was only one origin per chromosome. The replicative complexes present at the two forks could segregate the helix containing the Watson strand away from the other helix containing the Crick strand. Thus, initiation of replication (S) and segregation (M) could have been the same process, which was controlled by the same mechanism involving a primitive CDK. As eukaryotic cells became more complex with larger amounts of DNA, they required multiple origins for replication and chromosome condensation for mitosis. As a consequence, the two events S and M had to become distinct. However, the same CDK would still be initiating both events, with low levels initiating S and higher levels M. Such evolutionary scenarios are obviously speculative but provide at least a tenable explanation for why CDKs have such a central role to play in the regulation of such different events as S-phase and mitosis during the cell cycle.

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