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# Review

# Regulation of the Eukaryotic Cell Cycle

P. Nurse

Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A 3PX, U.K.

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## INTRODUCTION

THE CELL cycle is the process by which a cell ensures its correct reproduction, and is central to the understanding of all life. For a cell to survive, it is essential that at division it receives a full complement of all the components necessary for its survival. Of particular importance is the hereditary material DNA, which needs to be replicated and segregated in each cell cycle. Two events, common to all eukaryotic cell cycles, ensure that this occurs. These are the S-phase, when chromosomes replicate, and the M-phase or mitosis when the replicated chromosomes segregate into the two newly divided cells. Both of these events are subject to controls which regulate the onset of S-phase and mitosis. How are these events coordinated with one another. Why is it that if cells fail to replicate their DNA or have damaged DNA, they delay mitosis until these difficulties have been dealt with? Or why do cells which have not yet completed mitosis not undergo an extra round of S-phase, which would lead to genomic instability and changes in ploidy? These problems are of importance in understanding how the cell cycle is controlled.

## CELL CYCLE MUTANTS

The organism used for cell cycle research is the fission yeast *Schizosaccharomyces pombe*. This is a simple unicellular eukaryote related, but not closely related, to the more familiar budding yeast. *S. pombe* is useful as a model system for genetically investigating the cell cycle. It is easy to isolate mutants altered in cell cycle progression which define *CDC* (cell division cycle) genes. These genes can be cloned by complementation, that is, selecting for DNA fragments which rescue the mutant function because they contain the relevant gene. In this way it is possible to move from the more abstract analysis provided by classical genetics to the concrete analysis provided by molecular biology and biochemistry. This allows the identification and characterisation of the molecular components involved in the process regulating cell cycle progression.

Many cell cycle mutants are temperature sensitive which, when incubated at the restrictive temperature, are unable to divide. These mutant cells cannot divide but do continue to grow and produce highly elongated cells. Such mutants define genes that are necessary for proper progression through the cell cycle. However, they do not necessarily reveal those functions that are important for control. The approach discussed below has been used to identify such controlling genes.

At this stage it may be useful to make use of a metaphor, such as the operation of a car. If you want to identify what components are important for making a car go (that is what is required for a cell to divide), one approach is to take components away one at a time and see if the car now stops. For example, if the wheels are taken off or the engine is taken out the car will not work. However, a car is not controlled by taking its wheels or engine away. A car is controlled by more subtle means; for example, speed is regulated with the gear box or the accelerator. This means if the controls regulating speed are to be identified then 'mutants' which change the speed of the car must be found which will identify components, such as the gear box or accelerator. The equivalent cells will change the rate of cell division. If a cell is advanced through the cell cycle by accelerating the rate of division, the consequence is that the cell divides prematurely before it has grown to its normal size. Such mutants identify functions that are rate limiting for cell cycle progression, and not simply necessary for cell cycle progression. They were originally isolated in Scotland, and were called *wee* mutants (the Scottish term for small) [1].

## DISCOVERY OF CYCLIN-DEPENDENT KINASES

This type of mutant analysis first identified the *cdc2* gene as a crucial gene in regulating cell cycle progression in fission yeast [2]. The *cdc2* gene functions at the G2 to M transition and two different sorts of mutations were used to define how it worked. The *wee* mutants in the *cdc2* gene activated the *cdc2* function prematurely so that G2 was

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shortened and cells went into mitosis early. The *cdc* mutants in the *cdc2* gene knocked out the *cdc2* function completely so that the cells never went into mitosis, and elongated cells were formed. Furthermore, there was another unexpected property associated with *cdc2*, which was that it was also required late in G1 for the onset of S-phase.

It was discovered that the *cdc2* gene encoded a 34 kDa protein kinase [3] and, together with *CDC28* in the budding yeast initially described by Lee Hartwell, these were the first cyclin-dependent kinases (CDKs) identified. Cyclin-dependent kinases consist of a catalytic subunit, usually of 34 kDa, complexed with a cyclin component. There are three levels of regulation. First the association with cyclin is necessary for protein kinase activity, and cyclin degradation leads to inactivation of the kinase; secondly, protein phosphorylation of a tyrosine in the active site of the enzyme inhibits activity; thirdly regulation is also possible via specific cyclin-dependent kinase inhibitors. These inhibitors, which have emerged in the last few years, include p15, p16, p21 and p27. All three modes of regulation are important and will be discussed below.

## *cdc2* REGULATION OF MITOSIS

During the G2 period of the fission yeast cell cycle, the mitotic form of the CDK is formed by a complex of the *cdc2* protein kinase with the *cdc13* mitotic B cyclin. This complex is regulated by the *wee1* protein kinase, which phosphorylates tyrosine 15 and switches the kinase off, and the *cdc25* tyrosine phosphatase, which removes the phosphate and switches the kinase on to bring about mitosis. During G2, there is an accumulation of the *cdc13* B cyclin which forms a complex with the *cdc2* kinase. Because it is phosphorylated on tyrosine 15, activity is kept at a low, but not zero level. At the onset of mitosis, this phosphate is removed, activating the kinase to high levels bringing about mitosis. The *cdc2* protein kinase phosphorylates a variety of substrates required for the process of mitosis. To exit mitosis, the cyclin is degraded, inactivating the kinase, and the cell re-enters interphase and begins the next cell cycle [4].

## *cdc2* REGULATION OF THE S-PHASE

As already indicated, a second role for *cdc2* is at the onset of S-phase. At this stage of the cell cycle, the *cdc2* kinase is complexed with a different cyclin encoded by *cig2* which is required for the G1–S transition. This kinase becomes activated early in the cell cycle and brings about the onset of S-phase, but its regulation is not understood.

Our approach for looking at the control over S-phase was very simple. When a cell completes S-phase it goes into G2 and does not undergo another round of S-phase until mitosis is completed, when the cell enters G1 of the next cell cycle. One way to investigate the controls which bring about S-phase is to look for mutants, or cDNAs, which will promote S-phase from G2 leading to rereplication. What this does is to identify the controls that restrain onset of S-phase until mitosis is completed, and also identifies those functions which are able to bring about the onset of S-phase. It provides a powerful way of identifying the rate-limiting steps for the onset of S-phase, just as the *wee* mutants identified the rate-limiting steps for the onset of mitosis.

Mutants were selected that would undergo rereplication, by screening for cells which increase their ploidy. Only a few mutants were identified from a very extensive screen. Unexpectedly, these mutants identified the genes *cdc2* and *cdc13*; the mutations were particularly potent, completely eliminating the *cdc2/cdc13* kinase activity [5]. They not only blocked the onset of mitosis but were also able to induce an extra round of DNA replication. This led to the simple hypothesis that the *cdc2/cdc13* mitotic CDK, which in G2 is activated at a low level, has a double role. Not only is it involved in the initiation of mitosis, but at the same time it inhibits the onset of the S-phase. If the kinase is destroyed in G2, not only is mitosis blocked but the S-phase is also allowed to proceed. The reason why this was an attractive hypothesis was that when a cell exits mitosis the cyclin is destroyed, simultaneously destroying the inhibitory signal allowing the cell to undergo S-phase again.

Two experiments demonstrate this. In the first experiment, the *cdc13* gene was deleted [6]. As cells deleted for *cdc13* grow, they accumulate in G2 because they cannot undergo mitosis. Then, after a short delay, they re-enter G1 and undergo another round of S-phase. They undergo repeated rounds of DNA replication leading to a massive increase in DNA ploidy. This shows that if the mitotic kinase is destroyed completely, repeated rounds of DNA replication are induced. The second approach was to overexpress an inhibitor of the *cdc2* kinase, encoded by *rum1* (for replication uncoupled from mitosis). When *rum1* is switched on to high levels, it induces overreplication. Purified *rum1* protein is a potent *in vitro* inhibitor of the *cdc2* mitotic kinase, and when *rum1* is overexpressed in cells, it specifically associates with the mitotic kinase and inhibits its activity [7]. This shows that when the *cdc2* mitotic kinase is inhibited, multiple rounds of replication are induced and lead to the following simple model. A cell which is in G2 has a low level of the *cdc2* mitotic kinase, preparing the cell for mitosis. It has two roles. One is to prepare for mitosis and the second is to inhibit a further round of DNA replication. It is acting as a master switch not only regulating onset of mitosis, but also inhibiting S-phase. When the cell exits mitosis, the level of the kinase is reduced, the inhibitory activity is lost and the cell is now able to undergo S-phase.

How might *cdc2* inhibit replication? This is not known, but one possible candidate is a gene called *cdc18*. In the G1 phase of the cell cycle, there is a transcriptional control involving the transcriptional factor *cdc10*. It activates the transcription of a number of genes required for S-phase, a key one being the *cdc18* gene, which encodes a 65 kDa protein required for the initiation of DNA replication [8]. If this gene is overexpressed to a high level, it is sufficient to drive the cell into S-phase leading to rereplication. This is an important observation because although much is known about the process of DNA replication, it is not known what controls the initiation of replication. *cdc18* has several *cdc2* phosphorylation sites and so a very simple hypothesis would be that the *cdc18* protein is kept inhibited by the *cdc2* kinase, and therefore cannot initiate S-phase.

## CHECKPOINT CONTROLS FOR MITOSIS

Another crucial part of cell cycle control, are the check point controls, which ensure that a cell will not undergo mitosis if S-phase is incomplete. If DNA replication is blocked with a chemical inhibitor, such as hydroxyurea, then the onset of mitosis is blocked. This control works through the *cdc2* mitotic kinase because mutants in *cdc2* can

undergo mitosis even when DNA replication is blocked. If chromosome replication is incomplete, the *cdc2* mitotic kinase is switched off by keeping tyrosine 15 phosphorylated, and this prevents the onset of mitosis. So how does the cell detect that chromosome replication is not complete? A crucial role may be played by the *cdc18* gene already discussed. Cells in which the *cdc18* gene is deleted are initially stopped at G1 because *cdc18* is necessary for the G1 to S transition. Later, mitosis occurs and cells appear with half the DNA content of a G1 cell. This cell can be explained if *cdc18* has two roles—the regulation of the onset of S-phase and restraining the onset of mitosis. Cells deleted for *cdc18* cannot undergo S-phase, but also cannot prevent mitosis. After a short delay, the cell undergoes mitosis and cell division, producing cells with only half the normal chromosomes. Other genes also behave in the same way including DNA polymerase α.

These data can be interpreted as follows. Just before the onset of S-phase DNA replication, complexes are formed which require a few gene functions including *cdc18* and DNA polymerase α. The cell monitors these complexes and uses their presence as a marker that the cell is in S-phase and mitosis should be blocked. When DNA replication is complete and the complexes are depleted, mitosis is no longer blocked. Cells lacking *cdc18* or DNA polymerase α fail to generate the complexes, and so are unable to block mitosis even though DNA replication has not been completed [9].

## THE RELEVANCE OF CDKs TO CANCER

This review has focused on data obtained from the fission yeast *Schizosaccaromyces pombe*, but much that has been learnt in this simple eukaryote is also of relevance to Metazoa including human cells. The most striking example of this is the universal occurrence of CDKs and their role in cell cycle control. The human equivalent of the fission yeast *cdc2* gene has been cloned by complementation. An expressing human cDNA library was introduced into a temperature-sensitive *cdc2* yeast mutant and cells which could grow and divide at the restrictive temperature were selected. These had taken up the human *cdc2* gene which could substitute entirely for the fission yeast *cdc2* gene because the two genes were so similar. This experiment indicated that basic aspects of cell cycle control are highly conserved from yeast to humans and that study of these controls in convenient model systems will be informative about the analogous controls in human cells.

In mammalian cells there is a family of CDKs comprised of different catalytic enzymes and cyclins. These various complexes act at different stages of the cell cycle including the onset of S-phase and mitosis, as in yeast. They are also likely to be involved in blocking mitosis until S-phase is complete and ensuring that there is only one S-phase in each cell cycle. Given these similarities, what is the significance of this work for cancer? Two points can be made. The first is obvious. Cancer cells undergo unrestrained proliferation which means they have to undergo repeated cell cycles. It will be important to understand how the basic cell cycle elements are altered in cancerous cells, and their newly discovered elements provide a novel set of targets for intervention. Reducing the ability of cancerous cells to undergo major cell cycle transitions, such as onset of S-phase or mitosis brought about by the CDKs and their related control systems, may be useful in reducing cancer growth. The second point is more subtle. Cancerous cells are often genomically unstable and this instability may play an important role in generating the genetic changes which are prerequisite for cancer. We have seen that the CDK system is central to maintaining genomic stability by controlling ploidy and by preventing cells with only partially replicated DNA from dividing. Alterations in the core CDK elements may be necessary to generate genomic instability which could be a crucial step on the way to developing cancer.

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