The Nobel Prize in Physiology or Medicine 2001

From Yeast Genetics to Cancer Biology

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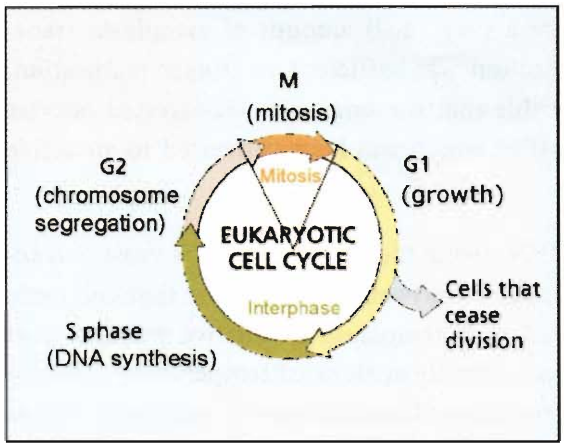
The cell theory proposed in 1838 by Theodor Schleiden and Jacob Schwann had two main tenets. One, every living organism is composed of one or more basic building blocks, cells, and two, new cells can arise only by the division of pre-existing cells. Thus, more than a century and a half ago it was apparent to biologists that cell division is the only path to cellular immortality. The complex sequence of events that produces two more or less indistinguishable daughter cells from the parent is the *cell division cycle* or the *cell cycle*. The task of dividing a cell is not as easy as it appears. It involves four things: the cell must grow (G1), replicate its DNA (S), segregate its chromosomes into two identical sets (G2) and then divide, that is, undergo mitosis (M) (*Figure* 1). A cell is faced with a number of problems if it wishes to produce two genetically identical daughter cells. These can be broadly listed as follows. Firstly, there is the completion problem, meaning that the cell has to follow a strict sequence of events in a linear fashion and ensure that the completion of one event causes the next. This guarantees that all the necessary events would have occurred before a daughter cell is born. Secondly, there is the alternation problem: events have to alternate with each other in a cyclic fashion such that no phase of the cycle repeats itself without the intervening occurrence of other phases. Leland Hartwell, Paul Nurse and Timothy Hunt were awarded the Nobel Prize in Physiology or Medicine for 2001 for their discoveries of key regulators of the cell cycle.

The logic of the cell cycle became first apparent, thanks to cell fusion experiments carried out with mammalian cells by Johnson and Rao in 1970. When cells from different phases of the cell cycle were fused to M phase cells, it was revealed that the M phase was in some sense dominant to other phases. Fusion

Keywords
Cell cycle, phase cells, *CDC* 28.



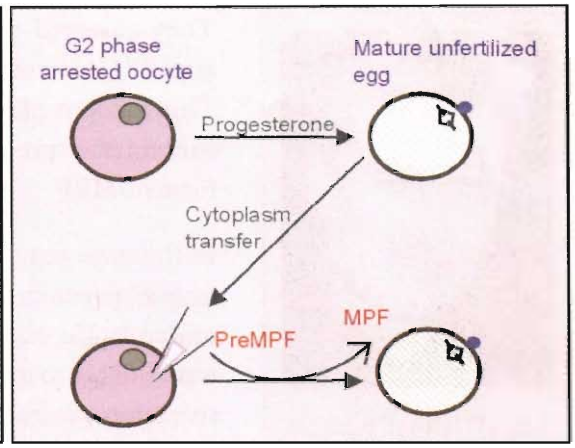


Figure 1 (left). The eukaryotic cell cycle comprises four distinct phases, each associated with a crucial function related to cell division. The G1, S and G2 phases constitute interphase during which growth, chromosome replication and segregation occurs. Mitosis gives rise to two genetically identical daughter nuclei.

Figure 2 (right). Discovery of MPF in frog oocytes by Masui and Markert (1971). Oocytes induced to mature into unfertilized eggs contain a cytoplasmic factor that can bring about maturation of G2 phase arrested oocytes. (Adapted from The Cell Cycle by Andrew Murray and Tim Hunt)

between G1 and S phase cells resulted in S phase cells inducing the G1 cells to replicate their DNA. The S phase cells did not enter mitosis until the G1 cells had completed replication. This showed that there were feedback controls and indicated a possible solution to the completion problem. Fusing S phase cells with G2 cells yielded a surprise. S phase cells were unable to induce replication in G2 cells and besides, there was a delay of entry into mitosis by G2 cells. This showed the presence of feedback controls or 'check points', a word later coined by Hartwell. It also provided a solution to the alternation problem by way of a 'block to re-replication'. Both the logic underlying cell cycle progression and the existence of mechanisms to ensure its fidelity were revealed by this experiment. A year later, in 1971, Masui and Markert set the stage for what became a boom in cell cycle research. Working with frog oocytes, they identified a cytoplasmic factor present in progesterone-induced mature eggs which could cause an oocyte that was arrested in the G2 phase to divide and mature into an unfertilized egg (*Figure* 2). They named this factor the maturation promoting factor (MPF).

Glossary:

Mitosis somatic cell division; process in which chromosomes duplicate and segregate during cell division.

Feedback control - the return of information; the result of the process reverses or shuts off the process.

Spindle a structure consisting of microtubules that helps in the alignment and movement of chromosomes during cell division.

Phosphorylation - the metabolic process of addition of phosphate to an organic compound.

Leland Hartwell works at the Fred Hutchinson Cancer Research Centre, Seattle, USA where he is the Director. He pioneered the use of *Saccharomyces cerevisiae* genetics to define the cell cycle and to understand its control. He identified the 'start' gene which controls the first step of the every cell cycle. He elucidated the genetic logic of cell cycle progression and identified and introduced the concept of checkpoints.

They observed that a very small amount of cytoplasm transferred by microinjection was sufficient to trigger maturation. This made it plausible that the immature G2-arrested oocytes contained a 'pre-MPF' which was later converted to an active form of MPF.

In the same year, 1971, using the common baker's yeast *Saccharomyces cerevisiae* Leland Hartwell identified the first cell cycle mutants. He started with temperature-sensitive mutants that were unable to grow normally at elevated temperatures (restrictive temperature), but showed normal growth and physiology at lower temperatures (permissive temperature). The use of temperature sensitive mutants is an approach that has proved of immense help in analyzing mutations which are lethal for a cell. The mutants displayed aberrant cell morphologies (shapes) at the restrictive temperature. Working with them, Hartwell realized that upon shifting to the restrictive temperature their growth stopped (arrested) at a particular stage (*Figure* 3). Luckily, in *Saccharomyces cerevisiae* the cell morphology can be very well correlated with the cell cycle phase. The observations hinted at a cell cycle defect in these mutants. Hence they were called 'cell division cycle' (*cdc*) mutants. Working with mutants

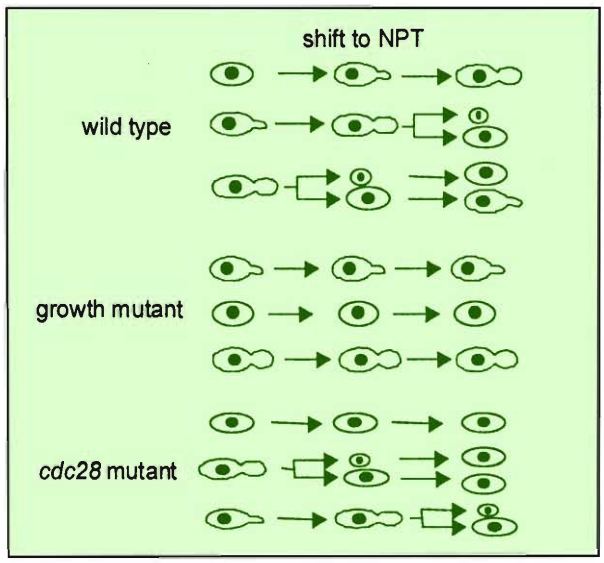
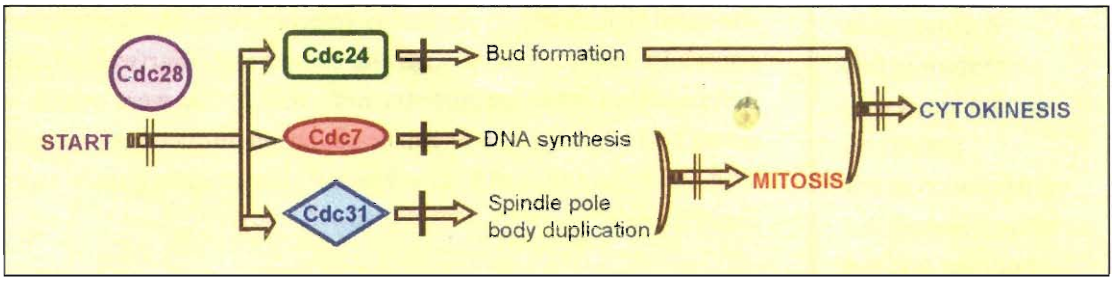


Figure 3. Cell division cycle mutants isolated by Hartwell in 1970, showed an arrest at a specific stage of the cell cycle upon shift to NPT (non permissive temperature). This feature helped identify these mutants as opposed to those with defects in growth and metabolism.



helped elucidate the genetics of cell cycle progression (*Figure* 4). In the same yeast Hartwell observed that one of his mutants, named *cdc*24, arrested in an unbudded state, but DNA synthesis and spindle pole body duplication occurred normally. The *cdc*7 mutant showed no DNA synthesis but bud formation and spindle pole body duplication took place normally. Similarly, in the *cdc*31 mutant, budding and DNA synthesis proceeded in the absence of any spindle pole body duplication. But none of these mutants underwent mitosis. This showed that early events of the cell cycle depended on its proper progression as a whole; meaning that the various events were interdependent. Among the more interesting of these mutants was the *cdc*28 mutant. In this mutant, none of the events associated with start of the cell cycle like DNA synthesis, bud formation and spindle pole body duplication occurred at the restrictive temperature and the cells arrested in G1. Hartwell called *CDC*28 the 'start' gene. This led to the concept of *start* – a point in the cell cycle where the commitment to undergo next round of the cell cycle is made. A stage similar to *start* in the budding yeast cell cycle also exists in

Figure 4. Use of cdc mutants to map cell cycle events. Hartwell used many such mutants to analyse their phenotype and developed a schematic for the interaction of various processes in the cell cycle.

Paul Nurse works at the Imperial Cancer Research Fund, London, UK and is the Head of ICRF. Using the fission yeast as a model, he identified and characterized the mode of function of the *CDC*2 gene. He showed the strong conservation of this gene from humans to yeast, thus demonstrating the remarkable conservation of this mechanism. He then elucidated the regulation of the activity of this protein and its role in cell cycle phase transitions. He has received the Gairdner Foundation International Award, The Albert Lasker Basic Medical Research award, which he shared with Hartwell and Masui.

A remarkable feature of this mutant locus was the strong conservation of the gene between the budding and the fission yeasts.

the mammalian cell cycle and is referred to as the 'restriction point'. In 1988, working with the same yeast, Hartwell identified radiation sensitive mutants (*rad*9, *rad*52) that were unable to arrest their cell cycle and repair radiation induced mutations. This led to the idea of a 'checkpoint' that ensures precision in the cell cycle.

In the same decade, Paul Nurse working with the fission yeast *Schizosaccharomyces pombe*, looked for mutants that were temperature-sensitive for cell division but not growth. Analysis of the DNA content and DNA synthesis at the restrictive temperature helped classify these mutants into the G1, S and G2 phases on the cell cycle. A mutant, *wee*1^{ts} ('wee' meaning tiny in Scottish), produced small cells, about half the size of wild type cells. This showed that cell size and cell division were linked. Nurse found that *wee*1 normally delays progression through the cell cycle until the cells have reached a critical threshold size. Another mutant, perhaps the most interesting one that Nurse characterized, was *cdc*2^{ts}. Most *cdc*2 mutants were recessive to the wild type and produced long cells at the restrictive temperature. However, one allele, *cdc*2-3*WD*, showed opposite effects. It was dominant to the wild-type cells and the cells entered mitosis at a smaller size. It was intriguing that the same locus showed two dramatically opposite mutant phenotypes. Analysis revealed that the *CDC*2 gene encodes a protein that induces mitosis. Its absence leads to the formation of long cells and an excess of its activity results in small cells.

A remarkable feature of this mutant locus was the strong conservation of the gene between the budding and the fission yeasts. In 1982, Nurse reported that the fission yeast mutant *cdc*2 isolated by him could be rescued by the *CDC*28 gene of the budding yeast. Thus, the fission yeast *cdc*2 was equivalent to the budding yeast *cdc*28 or the 'start' gene isolated in 1971 by Hartwell. This was the first report to show a strong conservation of key components of the cell cycle. Analysis revealed that Cdc2 belonged to a group of proteins called protein kinases which bring about phosphate transfer to target proteins. This can lead to loss or

Tim Hunt works at the Imperial Cancer Research Fund, London, UK and is the Head of the Cell Cycle Laboratory. He identified cyclins, proteins that regulate the function of *cdc*2/28 like CDKs. He showed that cyclin levels fluctuate during the cell cycle with a dip at every mitosis caused due to their degradation.

gain of their function, thus providing a genetic correlate of the earlier observed changes in phosphorylation levels of proteins during cell cycle progression (see below). Later, in 1987, Nurse went on to show that not just the budding yeast, but a human *cdc*2 counterpart which he called *CDK*1 was able to rescue the fission yeast *cdc*2 mutant. This provided compelling evidence for the conservation of not just the mechanism, but also the molecules orchestrating the cell cycle in organisms separated by at least 500 million years during evolution. The *cdc*2/28 story raised the question of how the same gene could be acting at two different points in the cell cycle in two different yeasts and yet complement each other functionally? This was later explained on the basis of differences in the budding and fission yeast cell cycles. In the budding yeast *start* is a key checkpoint for regulation in response to cell size, nutrient and mating signals, whereas in the fission yeast entry into mitosis is the key point of cell size control. Closer examination revealed that *cdc*2 was also required at *start* in the fission yeast.

The discovery of cyclin provided a biochemical model for the oscillator controlling the cell cycle.

In order to identify molecules that play decisive roles in cell cycle transitions, biochemists were looking for proteins whose levels in the cell fluctuated periodically. The premise that protein *synthesis* – and not any other aspect of protein metabolism, – should be periodic turned out to be a retarding factor in this search. In 1983 Hunt identified a protein in extracts of Sea Urchin eggs that disappeared abruptly at the end of each mitosis and then gradually reappeared during the next interphase. It was appropriately named cyclin (*Figure* 5). Cyclins were later shown to be controlling every important transition in the cell cycle. Against expectation, the cyclin protein was synthesized throughout the cell cycle and degraded at the end of each mitosis, causing its disappearance in cell extracts. In an interview Hunt said that it was "a complete off the wall" experiment "of the desperate variety". The discovery of cyclin provided a biochemical model for the oscillator controlling the cell cycle. By then Murray and Kirschner had independently provided evidence for regular oscillations of MPF activity in frog eggs.

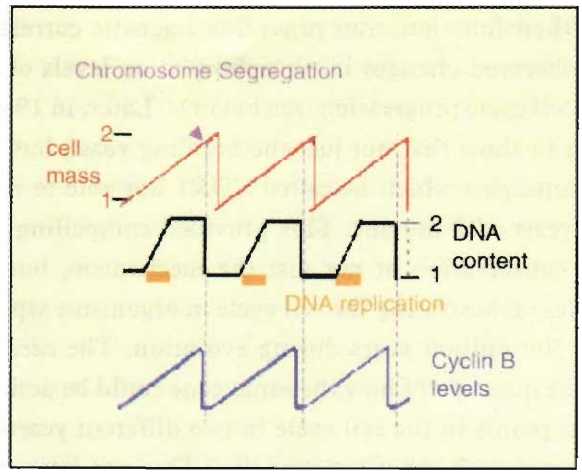


Figure 5. Cyclins levels oscillate during the cell cycle. Cyclin level peaks at mitosis with a sharp decline during exit from mitosis. This sharp dip in cyclin level is brought about by rapid degradation of the cyclin protein.

Also, by 1983 Hunt had shown that cyclin B is essential for mitosis. In an independent study, Murray and Kirschner had shown that the sea urchin cyclin B *m*RNA could alone drive the frog cell cycle *in vitro* even when the cell was depleted of other RNAs. This observation pointed to the conservation of this molecule too (like Cdc2/28).

Information generated from diverse systems projected two different views of the cell cycle with an apparent discrepancy in the organization of this fundamental process. Were Cdc2/28, MPF and cyclin identified in yeasts, frog and sea urchins all components of a single activity which controlled the cell cycle, or were they components of diverse convergent pathways, was the enigma. The purification of MPF in 1988 solved the problem. Lokha, Hayes and Maller purified and characterized MPF from frog egg extracts and saw that it possessed kinase activity. MPF was a dimer of two different subunits. The molecular weights of these two MPF subunits coincided with that of Cdc2/28 and cyclin B, but that had to wait immunological characterization to be confirmed. It was seen that antibody to yeast Cdc2 recognized the 34 kDa protein in the MPF complex and an antibody to the frog cyclin recognized the other 46 kDa subunit of the complex. Thus, the MPF recognised by Masui and Markert was nothing but a complex of the Cdc2/28 (identified by Hartwell and Nurse) and cyclin (which was Hunt's discovery).

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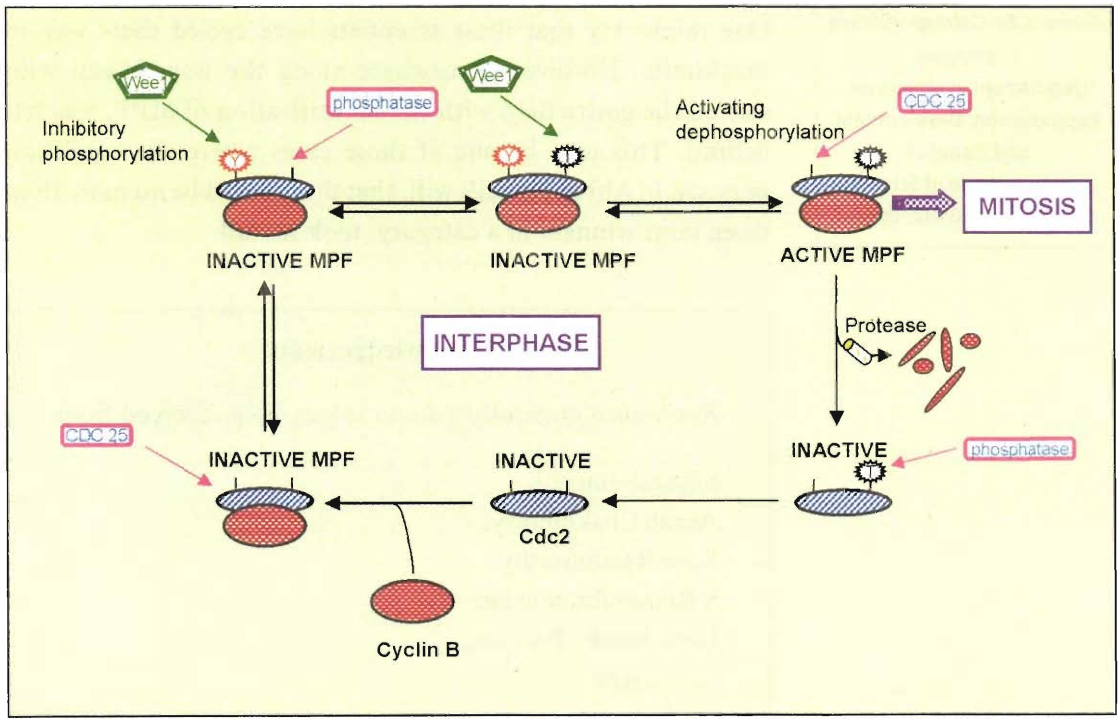
It became clear that geneticists, cell biologists and biochemists were addressing the same issue, handling the same molecules with different perspectives. Everything fitted in a picture when Nurse reported in 1990 that phosphorylation drives MPF function. Using molecular techniques and genetic analysis, he demonstrated that a particular tyrosine residue in the Cdc2/28 molecule was phosphorylated by Weel, product of *WEE*1 gene (identified by Nurse) and that this phosphorylation inhibited the kinase activity of Cdc2/28. He also demonstrated that *cdc25* (a long cell size mutant identified by him) encoded a phosphatase which removed the inhibitory phosphate at every mitosis, triggering the kinase activity of the Cdc2/28 complex during entry into mitosis (*Figure* 6). In 1989 Hunt demonstrated that cyclin degradation was essential for cells to complete mitosis and enter interphase, an observation that made the model more comprehensible. Cyclin functions as a regulator of the Cdc2/28 kinase activity and dissociation of the cyclin from the complex at mitosis renders the complex inactive.

Figure 6. MPF activity and its regulation during cell cycle. MPF is a heterodimer of Cdc2/28 and cyclin B. At the onset of mitosis, the phosphorylation state of the Cdc2/28 regulates its activity thereby deciding cell cycle progression. Degradation of the cyclin causes inactivation of this complex and exit from mitosis. During interphase the activity of the newly assembled complex is kept low due to inhibitory phosphorylation.



A unified picture of the cell cycle emerged with the components of MPF playing pivotal roles. MPF is a dimer of Cdc2/28 and cyclin B. At the onset of mitosis, the phosphorylation state of Cdc2/28 regulates its activity and decides cell cycle progression. Degradation of the cyclin causes inactivation of this complex and exit from mitosis. During interphase the activity of the newly assembled complex is kept low due to inhibitory phosphorylation. And that takes the cell to the next mitosis. Checkpoints integrate into this basic framework with pathways sensing extra and intracellular parameters regulating the phosphorylation status and thereby MPF activity.

The understanding of this fundamental event which sustains life and its perpetuation is fascinating just for what it is. In addition, research in this field has implications on human health: while controlled cell division causes growth, uncontrolled cell division causes cancer. Understanding the intricacies of the process will help design new therapies and may offer some help to treat a disease which affects one in six of us.

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One might say that these scientists have cycled their way to Stockholm. However, somewhere along the way, Masui who started the entire field with the identification of MPF, was left behind. This may be one of those cases where the condition imposed in Alfred Nobel's will, that there could be no more than three joint winners in a category, took its toll.

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