The Cell Cycle

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SYNOPSIS. I review recent advances in our knowledge of the eucaryotic cell cycle: the set of processes by which cells grow and divide. Genetic approaches to the cell cycle of somatic cells identified a pathway of events where the initiation of each event was dependent on the successful completion of the preceding event, as well as a single key gene, $cdc2$, that is required both at the beginning and at the end of the cell cycle. The alternative approach of studying the cell cycle biochemically in early embryos provided evidence for a cytoplasmic oscillator which alternated between mitosis-inducing and interphase-inducing states and identified the mitosis-inducing component as maturation promoting factor (MPF). These two very different views of the cell cycle initially seemed irreconcilable. However, a link between the somatic and embryonic cell cycles was provided by the recent discovery that the $cdc2$ protein is one of the components of MPF. In the embryonic cell cycle the activation of MPF and induction of mitosis is triggered by the accumulation of a protein named cyclin which becomes a component of MPF. Somehow, MPF induces the proteolytic degradation of cyclin, which in turn allows MPF to be inactivated and allows the cell cycle to pass from mitosis into interphase. The more complex cell cycle of somatic cells is probably derived from the embryonic cyclin-based oscillator by imposing a system of checks and balances on the accumulation and destruction of cyclin.

I also present some thoughts on the relationships between science and society, and comment on the way in which scientists describe their work to the lay world.

INTRODUCTION

The eucaryotic cell cycle is the ordered set of events between the birth of a eucaryotic cell and its division into two new cells. I will discuss recent work on the mechanism of the cell cycle that may provide a way of unifying the seemingly contradictory conclusions derived from two different experimental approaches to the cell cycle. I will consider the cell cycle as consisting of essentially two stages, mitosis and interphase. Conventionally interphase is divided into three periods: G1, the gap between the end of mitosis and the beginning of DNA replication; S, the period during which DNA replication occurs, and; G2 the gap between the end of S and the beginning of mitosis (Fig. 1).

One approach treats the cell cycle as a problem in genetics: How does each mitotic cell division produce a pair of cells that are genetically identical both to each other and to the cell whose division gave rise to them? The simple answer is that the genetic information was duplicated by replicating the chromosomes during interphase and then partitioned by segregating the replicated sister chromosomes from each other in mitosis. However the faithful transmission of genetic information depends not only on the accuracy with which the chromosomes are replicated and segregated, but also upon the coordination of these events. If the chromosomes have not been completely replicated before mitosis starts, cell division will produce daughter cells which are genetically different from each other.

STUDYING CELL CYCLE MUTANTS IN YEAST

Hartwell (1978) argued that the coordination of cell cycle events could be studied by finding mutants that prevent cell division by arresting cells at a specific point in the cell cycle. He chose to study bakers yeast, Saccharomyces cerevisiae, for two reasons: it is easy to study genetically and it divides by forming a small bud which grows and eventually separates from the mother cell. The latter feature means that the position of cells in the cell cycle can be determined simply by measuring the relative size of mother and bud (Fig. 1). Because cell division is an essential process, cell cycle mutations were found by looking for yeast

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strains that could grow at 20°C but not at 37°C. To distinguish mutants that affected the cell cycle specifically from those that arrested it as a consequence of general metabolic defects, Hartwell investigated what happened to cultures of mutant cells when they were shifted from 20°C to 37°C. At the time of the shift different cells in the population are at different points in the cell cycle. Only those mutants that caused all the shifted cells to proceed to a unique point in the cell cycle, and then arrest there, were classified as cell cycle mutants.

Hartwell and his colleagues then investigated the properties of these mutants, by determining which of the individual steps in the cell cycle, such as DNA replication or formation of a mitotic spindle, were defective in each of them. These investigators also studied the cell cycle by asking how strains which carried two different cell cycle mutants differed from strains that carried either mutant alone. They concluded that the yeast cell cycle was coordinated in a simple way: the initiation of each step in the cell cycle required the completion of the preceding step (Hartwell, 1978). Thus mitosis could not be initiated unless DNA replication had been completed, and chromosome segregation could not occur unless a mitotic spindle had been successfully formed (Fig. 1). This concept was named the dependent pathway to emphasize that the occurrence of cell cycle events depended on the completion of preceding steps.

The yeast cell cycle mutants also helped to define the points in the cell cycle where cells respond to the availability of nutrients in their environment and measure their size. A constant average cell size is maintained by coordinating the cycle of discrete events such as DNA replication and mitosis with the continuous increase in cell mass. If the time it takes a cell to double its mass is longer than its cell cycle time, each new generation of cells will be smaller than its parents; if the mass doubling time is shorter than the cycle time each new generation will be larger than its parents. In budding yeast the coordination between the continuous increase in cell mass and the dependent pathway of discrete events that comprise the cell cycle events occurs at a point early in G1 called START (Fig. 1). Cells cannot pass START unless they have reached a critical size and adequate nutrients are available; once they have passed START they are no longer sensitive to these two criteria.

The concept of the cell cycle as a dependent pathway derived from budding yeast...
has been reinforced by similar studies on another yeast, *Schizosaccharomyces pombe*, which diverged from budding yeast about 1,000,000,000 years ago. Unlike budding yeast, *S. pombe* divides by fission of a rod shaped cell into two cells of equal size, and it is therefore referred to as fission yeast. Essentially the same pattern of dependency of each cell cycle event on the completion of its predecessor is seen in both fission and budding yeasts. However, the fission yeast has a second point of physiological cell cycle regulation, at the transition between G2 and mitosis (M), in addition to regulation at START. Intriguingly, one cell cycle gene, *cdc2*, must be active in order to pass through both START and the G2/M transition (reviewed by Lee and Nurse, 1988). The equivalent gene in the budding yeast is named *cdc28*, and is required only at START. Nevertheless, the fission yeast gene will work in the budding yeast and vice versa.

**STUDYING THE CELL CYCLE OF CLEAVING EGGS**

A very different view of the cell cycle has been derived from studies on the first few cell cycles after fertilization of marine invertebrate and amphibian eggs (reviewed by Kirschner et al., 1985). These cell cycles have been popular objects of study because these large eggs can be regarded as cells that are specialized for cell division. The eggs of these organisms are available in large quantities, they divide rapidly and synchronously, and they do so without increasing in mass. The ability to divide rapidly without growing reflects two specializations: 1) the enormous stockpiles of all the components required for DNA replication and mitosis that have been laid down in the egg during oogenesis and 2) the suspension of the normal co-ordination between the discrete events of the cell cycle.

The embryonic cell cycles appear quite different from those of somatic cells (Fig. 2). In the embryonic cell cycle, interphase is occupied almost entirely by S phase: there is no G1 and little or no G2. More remarkably in some organisms, such as frogs, there is no evidence for any system of dependence within the cell cycle: inhibiting DNA synthesis or spindle assembly, or even completely removing the nucleus fails to arrest the cell cycle. There are two obvious alternative explanations for the lack of these feedback controls: either the synchrony of cell division in the early embryo provided by an inflexible cell cycle clock is more important than the safety net provided by feedback controls, or all the different feedback controls in the cell cycle are mechanistically related and suppressing the co-ordination of the cell cycle with cell size necessarily entails the suppression of the other feedback controls.

The lack of feedback controls in the early embryonic cell cycle suggested a conceptual framework where the behavior of the cytoplasm dictates that of the nucleus (Newport and Kirschner, 1984). Called the autonomous oscillator this framework states that some set of biochemical reactions produce an alternation between a cytoplasmic state where nuclei would be constrained to be in interphase and replicate their DNA, and an alternative state where the nuclear envelope is broken down and mitosis occurs. The observation that extracts from mitotic or meiotic cells could...
induce interphase cells to enter mitosis strongly suggested the existence of an activity that could induce the mitotic state. This activity, named maturation promoting factor (MPF) because it was first demonstrated by its ability to induce meiotic maturation of frog oocytes (Masui and Markert, 1971; Reynhout and Smith, 1974), has been highly conserved during evolution and is found in all eucaryotic cells that are in meiosis or mitosis (while being undetectable in cells that are in interphase). Purification and characterization of MPF have revealed that it is a protein kinase and that one of its subunits is the product of the \textit{cdc2} gene and its homologs in other organisms (Dunphy et al., 1988; Gautier et al., 1988). These observations suggest that the autonomous oscillator works by somehow activating MPF, which then induces a cascade of protein phosphorylation reactions that lead to nuclear envelope breakdown, chromosome condensation, the assembly of the mitotic spindle, and all of the other events that characterize the mitotic state. Subsequently the oscillator would activate an “MPF inactivase” that inactivates MPF and thereby allows the cell cycle to progress to the next interphase.

\textbf{Cyclin Synthesis and Degradation Controls the Embryonic Cell Cycle}

One clue to the mechanism of the autonomous oscillator was provided by the observation that protein synthesis was required for the entry into mitosis, but not for the subsequent exit and progression into interphase (Fig. 2). Because the key structural components for mitosis, such as tubulin, are provided from maternal stockpiles in eggs, it seemed likely that the protein synthesis requirement reflected the need to synthesize some special protein molecules that regulated the entry into mitosis. Furthermore, the fact that the egg was not synthesizing most structural components meant that the synthesis of such regulatory components might be visible against a very low background of other protein synthesis. The first candidate for such a protein was identified by T. Hunt and his colleagues (students in the Physiology Course in Woods Hole) who monitored the pattern of protein synthesis during the first three cell cycles of fertilized sea urchin eggs (Evans et al., 1983). Most of the newly synthesized proteins increased steadily in abundance throughout these cycles. But one protein, named cyclin, showed very suspicious behavior: its abundance increased gradually throughout interphase and then declined precipitously at the end of mitosis (Fig. 3a). This cyclical fluctuation was caused entirely by the sudden degradation of the protein at the end of each mitosis.

The kinetic behavior of cyclin made it an attractive candidate for a regulatory molecule that induced mitosis and suggested an attractive fantasy of how the cell cycle worked (Fig. 3b). Such a hypothesis invokes three components: MPF, cyclin, and an as yet hypothetical MPF inactivase. The inactivase, whose activity would be invariant throughout the cell cycle, would convert active MPF into an inactive form, while cyclin would stimulate the reverse reaction that activates MPF. Thus cyclin and the inactivase would work against each other to control the fate of MPF. At the beginning of interphase there would be little cyclin, the MPF inactivase would be the dominant activity, and MPF would therefore be maintained in its inactive form. As interphase progresses, cyclin would steadily accumulate so that eventually the ability of cyclin to activate MPF would exceed that of the inactivase to inactivate it. Now MPF would be activated and mitosis would ensue. To explain the escape from mitosis one can postulate that the activation of MPF induces the destruction of cyclin as well as all of the other events characteristic of mitosis. Once the cyclin has been destroyed, the MPF inactivase becomes dominant again, MPF activity disappears, and the cycle progresses to the next mitosis. This simple model of a biochemical oscillator can be thought as a flip-flop between two states, mitosis and interphase. Both states are unstable because they carry the seeds of their own destruction: in mitosis the seed
is the ability to accumulate cyclin, while in interphase it is the ability of MPF to induce its own degradation. In its simplest form, such a model would predict that cyclin is the only newly synthesized component required to induce mitosis and that cyclin degradation had to occur before cells could leave mitosis.

The first evidence in favor of a cyclin-based cell cycle oscillator came when purified clam or sea urchin cyclin mRNA was shown to induce meiosis after it was injected into frog oocytes (Swenson et al., 1986; Pines and Hunt, 1987). However, these experiments did not reveal whether the synthesis of other proteins was required or whether cyclin could also induce mitosis. We wished to test the role of cyclin in inducing mitosis by destroying all the mRNA in a frog egg and then adding back purified cyclin mRNA and asking whether mitosis was induced. These manipulations cannot be carried out in intact cells, so we followed the lead of Lohka and Masui (1983) and made extracts from frog eggs that would perform the cell cycle in vitro. The extracts contain very concentrated egg cytoplasm into which we add sperm whose plasma and nuclear membranes have been removed. The added sperm nuclei are initially very compact. Within 20 min they have swollen, acquired a nuclear envelope...
and started to replicate their DNA. At about 40 min the chromosomes condense and become visible, the nuclear envelope breaks down and mitosis occurs. Subsequently nuclei reform and a second and third cell cycle ensue (Fig. 4). When we examine the pattern of protein synthesis we find that a pair of cyclins accumulates in interphase and then disappears at the end of mitosis. These extracts appear to recapitulate all the key reactions of the cell cycle as it occurs in intact cells. Moreover, in extracts, as in vivo, protein synthesis is required during each interphase to induce the next mitosis (Murray and Kirschner, 1989).

Before such extracts could be used to determine whether cyclin was the only protein whose synthesis was necessary to drive the cell cycle, a method had to be devised that would destroy all the endogenous mRNA molecules in the extract. We did this by incubating the extract with low doses of RNase which destroys all the mRNA without affecting the function of either transfer RNA or ribosomal RNA. After this incubation we inhibit the RNase by adding RNase inhibitor, leaving an extract...
that is arrested in interphase because it is unable to synthesize proteins. However, this extract is still capable of translating added mRNA. When cyclin mRNA is added to such an extract the cell cycle is restored: cyclin accumulates during interphase, reaches a peak in mitosis and then is destroyed as the extract leaves mitosis and progresses into the next interphase (Fig. 5). Once more the cyclin accumulates until the extract again enters mitosis and the cyclin is once again destroyed. Thus the translation of cyclin mRNA in the absence of other mRNAs is sufficient to induce multiple cell cycles in an extract, indicating that cyclin is the only newly synthesized protein required not only for entry into, but also for exit from mitosis. In addition

the length of interphase decreases as we add more cyclin mRNA to the extracts, showing that at least part of the length of interphase in embryonic cell cycles represents the time required to accumulate cyclin to some level that is required for the successful induction of mitosis. Confirmation of the role of cyclin comes from experiments in which cyclin mRNA is specifically destroyed, causing the cell cycle to arrest in interphase (Minshull et al., 1989).
Embryonic Cell Cycle

WITHOUT FEEDBACK

WITH FEEDBACK

Failure to Finish DNA Replication

Failure to Assemble Spindle

Somatic Cell Cycle

Too Small, Inadequate Nutrients

"G1 CYCLIN"

Failure to Finish DNA Replication

Failure to Assemble Spindle

G1u G1c S G2 M G1u

To test whether cyclin degradation was required for the cell cycle to escape from mitosis, we made a mutant form of cyclin that could still induce mitosis but could no longer be destroyed by proteolysis. This mutant cyclin lacks the N-terminal 20% of the cyclin molecule, suggesting that this part of the protein carries signals that are used in the rapid degradation of cyclin that occurs at the end of mitosis. Introducing either this truncated cyclin protein or the mRNA encoding it into extracts drives the extracts into mitosis but does not allow them to exit from mitosis and enter the next interphase (Fig. 6). This experiment demonstrates that cyclin degradation is required to allow cells to exit from mitosis (Murray et al., 1989). The most recent evidence from workers in this field suggests that cyclin acts by becoming a subunit of the active MPF complex (Draetta et al., 1989; J. Gautier, T. Hunt, and J. Maller, personal communication).

Thus the fantasy of a cyclin based cell cycle oscillator appears to be true in outline: cyclin accumulation is sufficient to induce mitosis and the attainment of the mitotic state induces the destruction of cyclin which allows the exit from mitosis and the return to interphase. Can such a scheme also work in somatic cells that have complex feedback mechanisms that control their progress through the cell cycle?

Reconciling the Two Different Views of the Cell Cycle

How can we provide a link between the autonomous oscillator and dependent pathway views of the cell cycle? One way of providing this link is to imagine that a series of specific feedback controls work on the accumulation, activation and destruction of cyclin (Fig. 7). For instance a variety of circumstantial evidence suggests that cyclin is not competent to induce the activation of MPF until the cyclin protein has
been phosphorylated. Suppose that the activity that phosphorylates cyclin in somatic cells cannot be induced until DNA synthesis is complete. If this were true cells could not enter mitosis until all their DNA had been replicated. Similarly the destruction of cyclin that marks the beginning of the end of mitosis could be made dependent on the successful assembly of the mitotic spindle. It is intriguing that an entity that appears to prevent cyclin destruction does exist in unfertilized frog eggs (Masui, 1974). This activity, named cytostatic factor (CSF), prevents cyclin degradation, causing unfertilized eggs to arrest in metaphase of their second meiotic division. At the time of fertilization the interaction of the sperm with the egg causes a rise in the intracellular calcium concentration which inactivates CSF, thereby allowing cyclin to be degraded and the cells to enter the first mitotic interphase. Perhaps CSF is also the molecule that holds somatic cells in mitotic metaphase until some mechanical event signals the successful assembly of the mitotic spindle and induces an increase in the intracellular calcium concentration.

These types of checks and balances could explain the feedback controls that are used to co-ordinate the cell cycle with respect to the completion of DNA synthesis and spindle assembly in somatic cells (as well as in those embryos that retain these checks and balances). What about the regulation of the cell cycle in early G1 that occurs in response to cell size and nutrient availability? As I mentioned before, the cdc2 gene acts twice in the cell cycle: once at the G2/M transition and once at START. One attractive possibility is that the protein kinase activity and substrate specificity of the cdc2 protein is modified by the other proteins that it associates with. At the G2/M transition, the cdc2 gene product is a subunit of MPF and phosphorylates a set of substrates whose modification induces mitosis. It is tempting to speculate that at START, cdc2 associates with other proteins in order to phosphorylate a different set of proteins whose modification induces passage through START. In fission yeast, one of the accessory proteins required at the G2/M transition is the product of a gene called cdc13 (Booher and Beach, 1988; Hagan et al., 1988), which is the yeast cyclin homolog. Because the activity of cdc13 is not required at START, it is tempting to speculate that there is an as yet undiscovered member of the cyclin family, which we might call "G1 cyclin," that is responsible for activating the START-specific cdc2 protein kinase complex (Fig. 7). In the case of the G1 cyclin, the accumulation and/or activity of the protein could be regulated in some way by cell size and the availability of nutrients.

One clue that this is likely to be the case comes from studies of mating in budding yeast. Cells can only mate at one point in the cell cycle, just before START, and cells secrete mating pheromones that arrest the cell cycle of cells of the opposite sex at precisely this point. One interpretation of this arrest is that the mating pheromones somehow affect the feedback controls on START so that the cell must become infinitely large (or escape from the presence of the mating pheromone) before it can pass START. This interpretation has been substantiated by isolating mutants whose cell cycle cannot be arrested by mating pheromones. One gene, called DAF1, can be mutated to affect cell cycle control in exactly the predicted way (Cross, 1988). Mutants of this gene that have an increased activity of the gene product pass through START at a much smaller size than wild type cells, while mutants that inactivate the gene product pass through START at a much larger size than wild type cells. Remarkably when this gene was sequenced it turned out to be a highly diverged member of the cyclin family (Cross, 1988).

In conclusion, we can review how different viewpoints of the cell cycle came about and are now being reconciled. The concept of a dependant pathway arose from a genetic approach to studying the cell cycle, which was analogous to the earlier genetic dissection of metabolic pathways. In contrast, the idea of an autonomous oscillator came from studies on embryonic cell cycles. Without detailed biochemical data their proponents could believe that these two world views were essentially mutually exclusive. However the demon-
stration that the linchpin of the oscillator model, MPF, was composed in part of the product of the dependant pathway’s most famous gene, \textit{cdc2}, forced attempts at uni-

fication. These were fostered by the demonstration that the basis of the autonomous oscillator is likely to be the accumulation and destruction of a protein called cyclin.

Once biochemically fleshed out, the autonomous oscillator model could be modified to create a dependent pathway in order to explain the cell cycle of somatic cells. The genetic evidence that \textit{cdc2} functioned twice in the cell cycle suggested that cyclin and its relatives might do so too and such relatives have indeed been identified by genetic means in yeast. The apparent involvement of both cyclins and \textit{cdc2} in the two critical cell cycle transitions suggests that today’s more complicated cell cycles arose by an elaboration of a simpler cell cycle that had only one such transition. The continued interplay between the genetic and embryological approaches to the cell cycle has been crucial in working out this central problem in cell biology. Its history shows us that however snooty we may feel about our neighbors’ hypotheses, experimental approaches and world views, we usually profit by taking them seriously.

**THINKING ABOUT SCIENCE AS A WAY OF KNOWING**

Why is science valuable as a way of knowing? John Moore has suggested two reasons (Moore, 1986): to help people understand how scientific information is used in framing public policy decisions and to enable them to understand the conceptual framework that generates that information. I think it would help us to achieve those aims if we aired in public some of the limitations of science in these two areas.

Let us consider the use of scientific information in making policy decisions. The first limitation is that science itself cannot solve problems, it can only offer solutions to them. Decisions about the use of scientific remedies are made on the basis of the costs and benefits of adopting such remedies, and the assessment of costs and benefits inevitably reflects political considerations. One example of the interplay between science and politics, which John Moore previously referred to, is the question of acid rain (Moore, 1986). A significant fraction of the acid rainfall in New England and Canada is generated by sulfur and nitrogen oxides emitted from industrial smokestacks in the Mid West. This serious environmental problem could be greatly reduced by installing scrubbers that would reduce smokestack emissions of sulfur and nitrogen oxides. This solution has not been adopted because the cost of installing the machinery to reduce emissions has been deemed greater than the environmental benefits that reducing acid rain would bring. How were the costs and benefits analysed? By strictly political criteria, specifically, that the lobbying power of the industrial concerns that would bear the cost of reducing pollution, is greater than that of those who are concerned about the environment, which would receive the benefit.

Even the scientific information that is used to guide policy making can be influenced by political considerations. Consider the debate on the health risks of exposure to asbestos. For many years scientific studies on this topic that were funded by the asbestos industry disagreed sharply from those funded by federal grants or not-for-profit agencies. The industry-funded studies found little or no health risk, while those funded by other studies showed grave consequences of occupational exposure to asbestos. The companies involved refused to compensate their affected employees until years of litigation by dedicated public interest lawyers convinced the courts that there was indeed a causal link between asbestos exposure and a number of serious illnesses and that the industry had been aware of studies that demonstrated such a link.

Our second aim as science educators is to explain the conceptual framework that generates scientific information. When we do this we tend to present a monolithic and rather cold description of the scientific method: careful observation and the results of previous experiments lead to new hypotheses; these hypotheses make predictions that can be tested by new experiments or observations; and the results of such
experiments or observations either support or refute the hypothesis. At some level this general formulation encompasses the work of almost all scientists, but there are probably as many different versions of the scientific method as there are scientists. Some examples are: the relentless detective marshalling the evidence and following a series of inexorable clues, each revealed and tested by experiment, that lead to the correct hypothesis; the gambler, who on the basis of a few snippets of information and a hunch, constructs what could be called a fantasy as much as a hypothesis, and then tests it by one or two key experiments; the inventor, who is more interested in devising new techniques to solve problems than constructing grand hypotheses; and the careful observer who appreciates the significance of an unexpected observation and uses it to open up a new field. The practitioners of these different methods often disapprove of each other’s predilections. However, the continued advance of scientific knowledge depends on the interplay between their different ways of doing science.

As well as having prejudices about ways of doing science, scientists are often partial about the way they accept new findings. I think that attempting to create a unified and methodical description of science is partly responsible for some of the current confusion about the difference between fraud and difference of opinion in science. In the world we live in there is no unified set of standards; disagreement will be rife and its existence should not automatically call in the fraud-hunters. I think the public might like and understand us scientists better if we admitted our diversity, differences and prejudices rather than hiding them behind our white lab coats.

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