Basic Medical Research Award

Understanding the cell cycle

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Past, present and future

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The cell cycle has been the subject of intense investigation during two periods since Virchow's 1855 realization that cells only arise from pre-existing cells. At the turn of the century, microscopists and embryologists described the cytology of cell division in great detail, but could only speculate about the underlying mechanisms. In the late 1970s and 1980s, the flowering of molecular biology allowed cell biologists, biochemists and geneticists to join forces and better understand the working of the cell cycle in molecular terms. Their work has revealed that the basic processes and control mechanisms involved are universal in eukaryotes, and led to the view of the cell cycle as a highly regulated developmental sequence that brings about the reproduction of the cell. These studies have profited greatly from work on a surprisingly wide range of organisms, each with particular biological and methodological advantages. As a result, current understanding of the main events of the developmental sequence is good and in some cases very detailed.

The replication of chromosomes in S phase and their segregation at mitosis are of particular importance because they ensure that each daughter cell receives a full complement of the hereditary material. The machinery that brings about these essential cell-cycle events is composed of macromolecular assemblies that operate under cell-cycle controls. For example, during S phase, protein 'machines' generate the replication origins and forks required for duplication of DNA. To some extent, the operation of these machines is controlled locally; for example, errors of incorporation are corrected by built-in proof reading mechanisms. However, the operation of the machines is also monitored so that they communicate with other systems of the cell; for example, actively replicating forks inhibit entry into mitosis. The initiation of DNA replication normally depends on poorly understood, global controls that include sensors of cell mass and growth rate, as well as controls that prevent the re-replication of DNA until after the completion of mitosis. These global cell-cycle controls require monitoring and signal transduction circuits because they operate at a more extended level of time and space than is seen with local molecular interactions.

Two advances of recent years illuminate these issues. The concept of 'checkpoints' arose from the discovery of genes in budding yeast that are required for coordinating the progression of cell-cycle events when earlier processes have not been completed or when damage prejudicial to cell division has occurred. The cell-cycle block is not released until the defect has been corrected. As with other cell-cycle controls, checkpoints can also act at the level of local molecular interactions. This probably happens when DNA damage occurs during DNA replication, if the molecular machine processing the replication fork has components that detect DNA damage. Other times, the condition being checked may be very different from the event being blocked; for example, incomplete S phase or DNA damage during G2 both block a very different process, the onset of mitosis. Here, detection mechanisms that feed into a signalling pathway linked to the blocking mechanism must operate. Such a regulatory network means that successive cell-cycle events are not 'hard-wired' together, as would be true with steps in a metabolic pathway, and as a consequence they can be readily disrupted by mutation.

The second advance is the concept of rate-limiting steps that determine the onset of events like S phase and mitosis. This concept emerged from work on maturation promotion factor (MPF) and on fission yeast mutants that are prematurely advanced into mitosis, and eventually led to the identification of cyclin dependent kinases (CDKs) as regulators of progression through the cell cycle. Deregulating CDK activity by changes in phosphorylation, cyclin availability or CDK inhibitors all can drive cells prematurely into both S phase and mitosis. It is perhaps unexpected that closely related CDK activities can promote events as different as S phase and mitosis. This may mean that in the primeval eukaryote, cell-cycle progression was driven by a monotonic change in a single CDK, or that S phase and mitosis were initiated simultaneously.

Recently it has been shown that cell-cycle research has medical relevance. The shift from quiescence to an actively growing state is a pre-requisite for entry into the cell cycle in most cells, and is an essential transition in cancer. Also, because cancer is a somatic genetic disease, fidelity of genomic transmission is important for development of the disease and is dependent on the proper action of cell-cycle checkpoint controls.

Where is cell-cycle research going? Some straightforward developments in our present body of knowledge are necessary: understanding what changes to the mitotic cycle are required to bring about the altered cell cycle at meiosis; determining how cellular growth is initiated when cells move from quiescence into the cell cycle; understanding the molecular basis by which CDKs bring about S phase and mitosis; and establishing the importance of checkpoint defects for the development of cancer. Because such checkpoint defects may be common in cancer cells, they could provide important targets for therapy.

Beyond these immediate problems, the relative simplicity of the cell cycle and its universality make it a developmental sequence that in principle could be completely understood. This will require technology that allows the cell-cycle molecular machines to be studied in real time and space using microscopic imaging techniques such as fluorescence resonance energy transfer to follow molecular reactions in the living cell. Another important approach will be to exploit ongoing genome projects to try to identify all the genes required for the cell cycle.

Comparison of the budding and fission yeast genome sequences will soon be possible and will help in extending this type of analysis to multicellular organisms. Studying the cell cycle will be an ideal test of the methods being developed for post genomic functional analysis and for investigating the operation of developmental networks. This last problem may require new mathematical procedures for analyzing complex networks.

A proper description of the cell cycle will require understanding how global cellular characteristics interact with cell-cycle events and controls. The monitoring of cell mass, cell growth rate and cell-cycle oscillators or 'timers' have often been suggested as acting in cell-cycle controls, but how they might work is completely unknown. Spatial organization of the cell is also important; a good example of this is the generation of bipolarity required for chromosome segregation. However, the establishment of positional information within the cell and its interaction with cell-cycle events and controls still remain obscure. There are exciting times ahead, but if we are to be successful, proper attention must be paid to the biology of the cell cycle. Methods of molecular analysis have increased enormously in sophistication and rigor in recent years, but to be fully exploited for understanding the cell cycle they must also be complemented by good biological analysis and a proper 'feeling for the organism'—-in this case, the single cell.

Learning from the history of cell-cycle research

YOSHIO MASUI

The history of cell-cycle research is a demonstration of how molecular biology brought genetics and embryology—two sciences traditionally antithetical to each other—together as a single science. The animals that proved useful for genetics research (such as yeast) were of little interest to embryologists (who favored frogs, sea urchins and other marine invertebrates) and vice versa. Whereas geneticists were taught that the nucleus controls the cytoplasm by issuing genetic messages, embryologists had learned that the egg cytoplasm controls the nucleus by its determinants. Embryologists, basing their belief on the microsurgical experiments of the 1960s (such as nuclear transplantation, cytoplasmic dissection and cell fusion), were quite sure that the oocyte and egg cytoplasm controlled the nuclear activities associated with embryonic cell division and differentiation[1], but it was not until the late 1960s that they began to investigate the cytoplasmic substances responsible for cell divisions. When geneticists discovered cell division control (cdc) genes in yeast[2], embryologists did not know how to accommodate this remarkable discovery into their own work, as yeast cells lacked the chromosome condensation activity that the embryologists found in frog oocytes.

Embryologists reported that frog oocytes (even enucleated oocytes) stimulated to mature with progesterone showed chromosome condensation activity in their cytoplasm before initiating meiotic divisions[3]. They also discovered a protein-like substance with this activity, called maturation-promoting factor (MPF)[4], in extracts from frog eggs. MPF was also found in starfish oocytes[5] (Fig. 1) and, later, in the blastomeres of frog embryos[6], and in human[7] and yeast cells[8].

Meanwhile, biochemists working with sea urchin embryos discovered cyclin, a protein that embryos synthesize and destroy in synchrony with blastomere cell-cycle division[9]. Shortly thereafter, geneticists determined the sequence of the yeast cdc gene (cdc2) and identified it as a protein kinase[10]. This was the first great success in applying a molecular biology approach to cell-cycle research, and soon biochemists had also cloned and sequenced one of the cyclin genes[11]. Embryologists, on the other hand, purified MPF, and found that it was also a protein kinase. It was shown to consist of two subunits[12]: one later identified as cdc2 and the other as cyclin B (ref. 13).

MPF was first known for its function, then as a protein and finally as a gene. In contrast, CDC28 (or cdc2) was first described as a gene with a known function and only later was the protein identified. Cyclin was first discovered as a protein, then as a gene and finally its function was known as a component of MPF (Fig. 2). Thus, no matter which aspect of a cellular event is investigated first, either a gene by geneticists, a protein by biochemists or a function by embryologists, all sides will eventually be revealed and integrated under the umbrella of molecular biology.

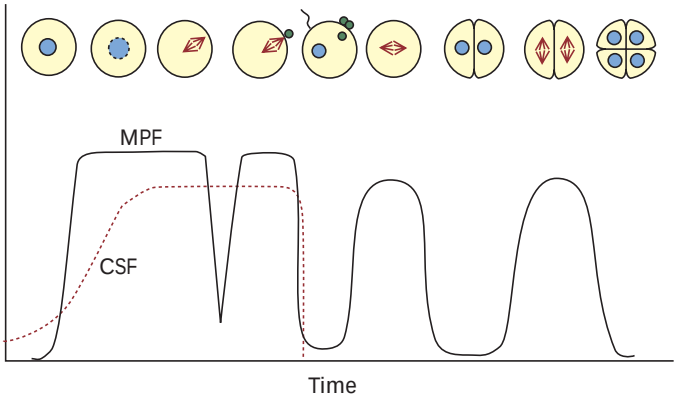


Fig. 1, Changes in maturation-promoting factor (MPF) and cytostatic factor (CSF) activities during meiotic divisions of oocytes and mitotic divisions of early zygotes. MPF activity is expressed as percentage of nuclear breakdown induced in oocytes injected with cytoplasm (or its extract) containing MPF or as histone H_1 kinase activity. CSF activity is expressed as percentage of blastomeres arrested at M-phase that had been injected with cytoplasm (or its extract) containing CSF.

The simpler the cell cycle, the easier the analysis of its control mechanism; much of the success of cell-cycle research is perhaps owed to the fact that early studies focused on the simplest eukaryotic cells. In yeast, unlike in multicellular organisms, the cell cycle lacks chromosome condensation and is unaffected by cell contacts and cell differentiation. In frog and marine invertebrate oocytes and zygotes, both meiotic and mitotic cell cycles progress without requirements for cell growth and external nutrients. Gene activities of the oocyte are turned off just before meiotic divisions, and those of the zygote are not turned on until the midblastula stage. Therefore, their cell cycles, which lack G1 and G2 phases and checkpoints, are totally controlled by the cytoplasm. The simplest cell cycle reveals the essential minimum of cell-cycle control. Unfortunately, differ-

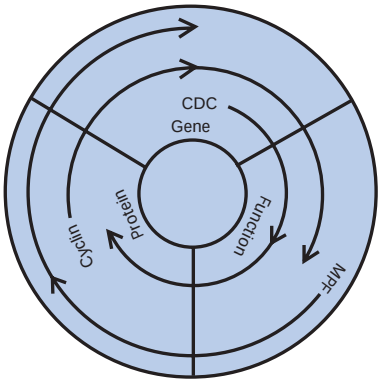


Fig. 2 The pattern of the progress in understanding cell division. MPF and its components were first discovered independently as unrelated entities. MPF was first found in the function domain, CDC first in the gene domain, and cyclin first in the protein domain. However, other aspects of these entities were revealed later in relation to each other (arrows): MPF in the protein domain, and finally in the gene domain; CDC in the function domain, and finally in the protein domain; cyclin in the gene domain, and finally in the function domain.

entiated cells of multicellular organisms have more complex cell cycles. These cell divisions are regulated not only by cytoplasmic growth and cell size, but also by cell contacts (for example, contact inhibition). Furthermore, they are conditioned by growth factors, nutrients, cell differentiation processes and more. For a cell to regulate the cell cycle in response to a variety of internal and external factors as such, the cell must have many sensitive checkpoint mechanisms effected by nucleocytoplasmic interactions through complex feedback systems.

We now find ourselves in a situation that seems similar to that in which physicists were when it became increasingly difficult to explain the complex behaviour of real gases using the ideal gas theory. To better understand the cell cycles of developed multicellular organisms, we may need to not only develop innovative research techniques but also revise the concepts that currently govern our thinking.

There are other challenges as well: we might, for example, ask whether we must further expand the ever-growing list of genes controlling the cell cycle. If so, we may soon find that we have moved beyond our ability to comprehend the whole subject we are studying. Indeed, this is what I fear most. To understand such a complex system we will have to develop a new approach that will allow us to integrate the multitude of signal transduction pathways that control the cell cycle. Mathematical modelling of the cell cycle[14] may serve this purpose provided that accurate quantitative analyses of molecular processes and cell behavior related to the cell cycle is possible, such that we can check the validity of the models.

Metaphors for the cell cycle

LELAND HARTWELL

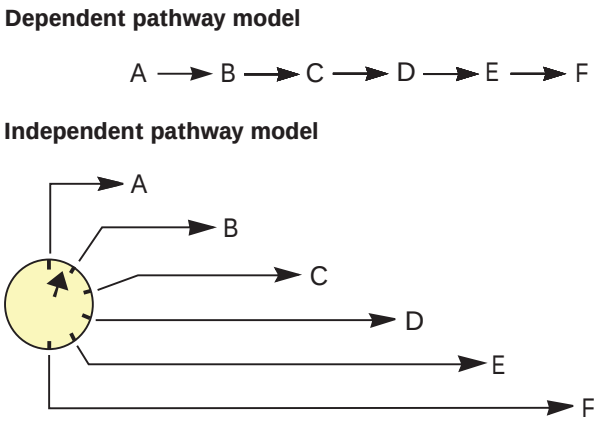
The cell cycle is an orchestrated series of events in which early events (such as DNA replication) must be completed before later events (such as sister chromatid segregation). Twenty to thirty years ago the central question seemed to be whether a conductor was needed to set the tempo and signal successive events or whether the cell functioned more like a jazz ensemble in which order derived from the participants themselves (see Fig.).

To a cytologist, the cell cycle seemed to be mostly a series of macromolecular assemblies. The complex structure of the chromosome is duplicated and the mitotic spindle obviously assembles and disassembles each cell cycle. Moreover, the ordered events of the cell cycle (DNA replication, centrosome duplication, spindle assembly and chromosome segregation) were cases in which early events provided the substrates for later events. An obvious paradigm existed for the intrinsic generation of order in a complex biological assembly process from elegant work on the morphogenesis of the bacteriophage T4 particle[15]: more than fifty proteins assemble to produce the phage T4 using only their intrinsic affinities.

The concept of the cell cycle as a pathway of interdependent assembly events was strengthened by analyses of temperature-sensitive mutants of *Saccharomyces cerevisiae*[16] and *Schizosaccharomyces pombe*[17] that each interrupted the cell cycle at a specific stage. An asynchronous culture of mutant cells would become synchronized at a particular event after a shift to the restrictive temperature, indicating that all events but one could occur at the restrictive temperature and that when a cell found itself unable to complete one particular event it did not attempt events that normally would have occurred subsequently. The nuclear pathway could be explained as a single sequence of dependent events with no need for an extrinsic director of the events.

However, a different view emerged from work on amphibian embryos. Enucleated eggs of *Xenopus* were found to contract with the same periodicity as cell division occurred in their nucleated counterpart[18]. This result indicated that a 'clock', located outside the nucleus, directed the cell cycle, and, even in the absence of DNA replication and mitosis, the cell was receiving periodic signals to initiate division. Earlier work had described cytoplasmic activities that induced chromosome condensation and nuclear envelope breakdown[3]. Returning to the musical metaphor, when the horn player failed to respond to the conductor's signal, the violins nevertheless picked up on cue.

Hence, the cell cycles of yeast and Xenopus seemed to be regulated in entirely different ways, and even the two yeasts *S. cerevisiae* and *S. pombe* had fundamental differences, with growth entraining the cell cycle in G1 in the former and in G2 in the latter. These apparent disparities were synthesized into a unified view when the most important cell-cycle regulatory components of *S. cerevisiae* (CDC28), *S. pombe* (cdc2), and *Xenopus* (maturation-promoting-factor) turned out to be cyclin-dependent protein kinases[19]. A unified view was embraced by all when it was shown that these genes as well as the human counterpart could substitute for one another. Many subsequent genetic and biochemical studies led to our current understanding of the essential regulators of the cell cycle as the cyclin dependent kinases whose activity passes cyclically through distinct stages by periodic cyclin transcription and degradation[20]. In the *Xenopus* embryo, this regulatory cycle is a self-regulating clock that orchestrates the other cell-cycle events, whereas in the yeasts and in metazoan somatic cells, the clock is entrained each cycle by growth and other requirements.



Two possible models for ordering cell-cycle events. In one, the dependent pathway model, early events provide the substrate for and trigger later events. In another, the independent pathways model, a central clock triggers successive events. (Adapted from ref. 16).

Why then did so many cell-cycle mutants of the yeasts arrest not just a single event of the cell cycle but all subsequent downstream events as well? Many gene products have now been identified that result in cell-cycle arrest when they are deficient. Many of them are indeed elements of the clock—mutations that inactivate the CDK, cyclins or components of the ubiquitin-mediated proteolysis. These mutations in the clock would be expected to arrest all cell-cycle progress. However, many of the mutations inactivate components of the peripheral machinery—such as DNA polymerase or tubulins—and these also prevent the execution of events downstream of their site of action. Eliminating the horn play does indeed silence the violins.

Understanding this behavior required the identification of an additional level of control. The performance of the cell-cycle machinery is monitored by surveillance mechanisms, checkpoints that prevent the cell from advancing to the next stage when there is a defect[21]. These surveillance mechanisms have probably evolved to provide cells time to repair damage or complete cell-cycle events (such as chromosome attachment to the spindle) before the cell progresses in the cycle to a stage in which irreversible damage would be incurred.

At one time it seemed reasonable to think of the cell cycle as an autonomous pathway of macromolecular assemblies. However, we now see it very differently. The central element seems to be a clock whose mechanism encompasses periodic transcription and degradation of the cyclin component of cyclin-dependent kinases that is entrained by growth, other extrinsic signals and surveillance mechanisms that interrupt progression when defects occur. It is a testimony to science that even though much evidence can be found along the way that is consistent with our preconceptions, eventually the weight of experimental results forces us to embrace new paradigms.

There are many aspects of the cell cycle that remain unclear (how growth is monitored, for example). Our delving into the details of cellular mechanisms has revealed a complexity that is bewildering. The concepts of gene regulation, self-assembly and molecular pathways, although valid on the microscale, no longer seem adequate to encompass the complexity we are seeing. Our current methods of genetics, biochemistry and cell biology can provide a list of components and limited insights into molecular mechanisms; however, new methods that permit real-time, dynamic visualization of molecular machines *in vivo* and that facilitate molecular analysis on a genome-wide scale. The themes we are beginning to hear more frequently have to do with networks, patterns and states, representing attempts to deal holistically with the complexity of the cell.

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