S Phase of the Cell Cycle

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In each cell cycle the complex structure of the chromosome must be replicated accurately. In the last few years there have been major advances in understanding eukaryotic chromosome replication. Patterns of replication origins have been mapped accurately in yeast chromosomes. Cellular replication proteins have been identified by fractionating cell extracts that replicate viral DNA templates in vitro. Cell-free systems that initiate eukaryotic DNA replication in vitro have demonstrated the importance of complex nuclear architecture in the control of DNA replication. Although the events of S phase were relatively neglected for many years, knowledge of DNA replication is now advancing rapidly in step with other phases of the cell cycle.

DURING THE S PHASE OF THE CELL CYCLE THE ENTIRE DNA content of the nucleus must be replicated completely and precisely in a period of a few hours. This is achieved by initiating bidirectional replication at multiple sites along each chromosome. A single replication fork traveling at the observed rate of 3 kb per minute would require about a month to replicate an average human chromosome containing 40 mm of DNA. Failure to complete replication within an S phase would lead to chromosome breakage at the next mitosis. Similarly, local reinitiation of replication within one S phase could also have adverse consequences. This phenomenon is only observed in rare special cases. Therefore, patterns of initiation within a single chromosome must be regulated spatially and temporally to ensure complete and precise replication within S phase.

Mechanisms that regulate replication within S phase must be versatile. The length of S phase can vary, not only between species (*1, 2*) but also between different developmental stages of the same species (*3*). Adult cells of *Drosophila melanogaster* replicate their DNA in an S phase of about 10 hours, whereas early embryos of the same species replicate in an S phase of less than 4 min (*3*). The length of S phase is determined by factors other than the time it takes to replicate a certain length of DNA.

It is easily forgotten that chromosome replication involves more than the replication of DNA. In addition, the complex architecture of the chromosome must be duplicated too. This must involve assembly of nucleosomes and chromosome scaffolds. Furthermore, in some cells it also involves copying specific patterns of gene activity and inactivity.

In this article we consider how eukaryotic chromosomes are replicated in S phase. For many years eukaryotic DNA replication has been neglected compared to eukaryotic transcription or prokaryotic DNA replication. However, the last few years have brought exciting progress on several fronts. Replication origins have been precisely mapped in yeast (*4–7*). At least two replicative DNA polymerases have been identified, characterized, and assigned likely roles at the replication fork (*8, 9*). Cell-free systems have been developed that have allowed the isolation of cellular proteins which are required for viral DNA replication and are thus good candidates for roles in cellular DNA replication too (*8, 10*). Initiation of DNA replication has also been achieved in vitro (*11–13*), under conditions indicating that the complex structure of the cell nucleus may be involved in regulating DNA replication in S phase.

Entry into S Phase

The signals that induce cells to proliferate are the subject of other reviews in this issue, but microinjection and cell fusion studies in the 1960s established that nuclei are induced to enter S phase by dominant cytoplasmic signals (*14, 15*). Thus S phase cytoplasm induces nuclei from nonproliferating cells to replicate their DNA. No species specificity has been observed for induction. Nuclei from one species are induced to replicate by S phase cytoplasm of all other species tested. Furthermore, when adult nuclei are injected into *Xenopus* eggs, they are induced to replicate in the very short times characteristic of early amphibian embryos (*14*). An interesting exception is seen by the failure of G_2 nuclei to replicate after fusion to S phase cytoplasm (*15*). In some way replicated nuclei are distinguished from unreplicated nuclei so that replication occurs only once in a cell cycle.

Patterns of Initiation

The length of S phase differs between species and between different developmental stages within a species (*1–3*), but within any particular type of cell S phase is remarkably constant in length. This means that there must be precisely determined patterns for regulating the events in S phase. Other lines of evidence reinforce this conclusion. For example, mitotic chromosomes that have been pulse-labeled with bromodeoxyuridine (BrdU) show broad bands of incorporation indicating that specific regions of the chromosome replicate at different times (*16*). Similarly, autoradiography of pulse-labeled DNA spreads ("fiber autoradiography") shows that clusters of replication units ("replicons") initiate synchronously, but that different clusters appear to initiate at different times (*1, 2, 17*).

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Further evidence that initiation patterns are precisely spatially regulated has been obtained more recently from studies of replication origins and optical studies of replicating nuclei.

Origins of Replication

Unlike most bacteria, eukaryotes initiate replication at multiple sites within the chromosome. Identifying and isolating these replication origins have been major priorities within the field for many years. Nevertheless, eukaryotic origins of replication have been remarkably elusive with the conspicuous exception of the yeast *Saccharomyces cerevisiae*.

In yeast a selective screening strategy yielded a subset of DNA sequences called autonomously replicating sequences, or ARS elements, which allow plasmids containing them to replicate extrachromosomally (*18*). DNA sequence analysis revealed a short conserved core consensus sequence in ARS elements (*19*), and both deletion and base substitution mutagenesis (*20–22*) revealed that this sequence is essential for autonomous replication of plasmids. Furthermore, flanking sequences were also important to maximum efficiency of replication. Autonomously replicating sequences were good candidates for cellular replication origins.

Two-dimensional gel mapping methods have been developed for determining the sites at which replication initiates (*4, 5*). These methods have confirmed that replication initiates at autonomously replicating sequences within intact yeast chromosomes (*6, 7, 23*) and have given the first glimpse at how eukaryotic replication forks are distributed with respect to DNA sequence. Several features have emerged from these studies. For example, not all ARS elements are used as replication origins. Within the tandemly repeated ribosomal RNA only a fraction of the potential origins are used within a given cell. In the case of the ribosomal repeat, the two replication forks that diverge from a single initiation proceed unequal distances. Replication initiates in the nontranscribed spacer, and the replication fork that moves against the direction of transcription pauses when it reaches the end of the spacer and meets the 3′ end of the nearest transcription unit. In contrast, the replication fork that moves in the same direction as transcribing RNA polymerases proceeds right through the adjoining gene and through subsequent spacers and genes until it reaches a fork that had paused near the next replication origin, as shown in Fig. 1 (*6, 7*). The power of the origin mapping techniques that produced these results is now clear. Their application to other systems is likely to produce a cascade of valuable information on patterns of DNA replication.

The search for origins of replication in higher eukaryotes has been less successful so far. Several candidate sequences have been isolated by a variety of methods (*24*), but their significance has been difficult to assess as there is a conspicuous lack of assay systems for replication origins in higher eukaryotes. Indeed, in at least one case a sequence that appeared to confer autonomous replication in mammalian cells was subsequently found to be replicating as integrated tandem copies in the chromosome rather than as an extrachromosomal plasmid (*25*). One possible explanation of the difficulty in obtaining autonomous replication of plasmids in mammalian cells is considered below.

Although replication origins have been difficult to isolate from mammalian cells, extensive evidence shows that replication is precisely regulated with respect to DNA sequences. One of the clearest examples is provided by studies of cells that have amplified copies of the genes for dihydrofolate reductase (DHFR).

When Chinese hamster ovary cells are selected for their ability to grow in the presence of the drug methotrexate, lines emerge that have amplified the DHFR gene many times. A line called CHOC 400 contains about 1000 copies of the DHFR gene arranged as tandem repeats within the chromosome. This high copy number has allowed origin mapping within the repeat, yielding clear evidence of a preferred initiation region (*26, 27*), which has now been cloned and sequenced (*28*). Furthermore, a second, more complex, mapping approach has recently confirmed this assignment (*29*). Precise identity of the nucleotides involved awaits development of assays for mammalian origins.

Another approach for mapping the initiation and progression of replication forks in a range of cultured cells has been developed and exploited by Schildkraut and colleagues (*30–32*). Proliferating cells are pulse labeled with the dense thymidine analog BrdU and subsequently separated on the basis of their DNA content and hence their position within S phase. DNA is extracted from cell populations at different stages in S phase, and it is fractionated on CsCl gradients to separate dense DNA, containing BrdU, from normal, light DNA. The dense and light DNA fractions from each cell cycle stage are then probed by hybridization probes for the specific genes under investigation to reveal the precise time of replication of specific genes. Several conclusions have emerged from this approach (*26–28*). First, within a given cell type individual genes replicate at defined periods in S phase. Second, many active genes replicate early in S phase. Third, the time of replication of a particular gene may vary between cell types depending on whether or not the gene is active. Fourth, some long linear arrays of contiguous genes replicate in their order in the chromosome. This has been observed for DNA lengths of 300 kb, suggesting that they might be replicated by a single replication fork progressing through this entire region.

Although there is abundant evidence that replication patterns are regulated spatially, there are exceptional cases that raise questions about the biological role of replication origins. When DNA is injected into unfertilized eggs of *Xenopus laevis*, it replicates under cell cycle control without requiring any specific eukaryotic sequence (*33, 34*). Thus, for example, bacteriophages λ, ϕx174, G4, and M13, and a range of prokaryotic plasmids injected into *Xenopus* eggs replicate multiple times in multiple cell cycles, yet only once in a single cell cycle. A similar pattern is seen when prokaryotic vector DNA is injected into the macronucleus of the protozoan *Paramecium* (*35*). These experiments do not show that eukaryotic cells replicate their genomes randomly without specific replication origins. However, they do show that specific replication origins are unnecessary either for the enzymes of replication in this type of cell or for the mechanism that couples replication to the cell cycle, ensuring that DNA replicates only once within any cycle. Why then are replication origins necessary in other circumstances? The answer to this question is not clear yet, but we have speculated (*36*) that it might be related to the lack of transcription during the stages of exceptionally rapid replication in early *Xenopus* embryos. The possibility that the role of specific replication origins is to coordinate DNA replication with gene transcription is considered in the final section of this article.

The Replication Fork

Once initiation has occurred, how do replication forks elongate the nascent chains? Much of the recent knowledge of eukaryotic replication forks, and the proteins involved there, comes from cell-free systems that replicate viral DNA in vitro. Simian virus 40 (SV40) has been the most instructive virus to date because its replication requires only one viral protein, namely, T antigen. Apart from T antigen, SV40 replication depends entirely on cellular proteins. Therefore, it offers a particularly good model system for analyzing the components of the eukaryotic replication fork (*8*).

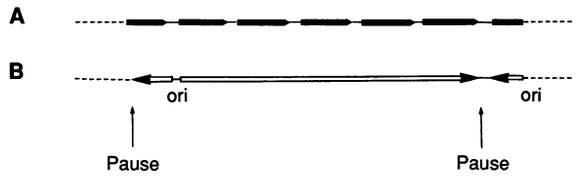

Fig. 1. Pattern of replication origins and replication fork movements in the tandemly repeated ribosomal RNA genes in yeast. The upper line (**A**) shows the alternating positions of transcription units (solid arrows) and nontranscribed spacers. The lower line (**B**) shows the sites of initiation (ori) and pausing of replication forks (pause). Only a subset of potential origins are used, and forks moving against the direction of transcription stop near the 3′ end of the transcription unit. From data in (*6*); also see (*7*).

Two DNA polymerases appear necessary for the replication of SV40 in vitro, DNA polymerase α and DNA polymerase δ. Polymerase α contains a primase activity in two of its subunits, suggesting involvement in the discontinuous synthesis of Okazaki fragments on the lagging strand (Fig. 2) (*37*). In contrast, polymerase δ lacks primase activity and there is evidence (*38*) that it is required for synthesis of the leading strand (Fig. 2). Involvement of polymerase δ in SV40 replication emerged when a fractionated factor required for replication in vitro was identified as PCNA (proliferating cell nuclear antigen). PCNA had previously been shown to be a cofactor of polymerase δ, greatly increasing its progress along the template ("processivity"). Direct confirmation that both polymerases are required for chromosomal replication comes from the yeast, *S. cerevisiae*, in which the equivalent polymerases to α and δ (called I and III in yeast) are both essential (*39, 40*).

Polymerase δ or III in *S. cerevisiae* is encoded by *CDC2*, but this is unrelated to the *cdc2* gene of *Schizosaccharomyces pombe* (*41*). The potential for confusion is increased severely because the unrelated products of these two genes bind two unrelated proteins that have both been called "cyclin" in the literature, one of which is now called PCNA. Use of the term "PCNA" instead of cyclin for the cofactor of DNA polymerase δ can help to limit the confusion caused by this coincidence.

Further fractionation of the SV40 system has also allowed the identification and characterization of other proteins involved in replication. Reconstitution of the replication system in the presence and absence of each protein and the use of specialized assay systems have identified the role of each factor and the stage at which it acts. One essential protein identified in this way was found to be a multisubunit complex of three polypeptides termed RF-A (*42*) or RP-A (*43*). This protein complex binds to both single- and double-stranded DNA but preferentially to single strands and is thought to function as a eukaryotic single-stranded DNA binding protein. With the use of an unwinding assay, which generates a characteristic product detectable by gel electrophoresis (form U) (*44, 45*), it has been shown that this protein is required after the formation of the initial complex between T antigen and the origin DNA. Its high affinity for single-stranded DNA suggests that it is needed to stabilize a single-stranded region for the correct positioning of the polymerase/primase complex at the origin for initiation of DNA synthesis. The same unwinding assay has also been used by Roberts and D'Urso (*46*), in conjunction with the technique of cell elutriation, to demonstrate that cellular extracts made from S phase cells are much more efficient in the production of the unwound form U than extracts made from other phases of the cell cycle. By using this as an assay they are therefore trying to purify this factor that they suggest may act as a cellular initiation factor.

Other work has identified a protein which, although not essential for replication in vitro, appears to stimulate the initial interaction between T antigen and origin DNA (*47*). This protein, termed RP-C, has since been identified as the catalytic subunit of the human protein phosphatase 2A (PP2Ac). In light of the recent plethora of phosphorylation/dephosphorylation reactions identified as being key events in cell cycle control (*48*), it will be interesting to identify the target of this phosphatase and its role in DNA replication.

A similarly named but quite separate protein RF-C has also been identified, which, like PCNA, is required for the coordination of leading and lagging strand synthesis of the replication fork (*49*). This protein, which is also a complex of several polypeptides, appears to show no enzymatic activities; it has been suggested that it acts as a "clamp" between α and δ polymerases. However, the unmasking of cryptic activities when cellular factors and polymerases interact would not be totally surprising; such activities may not be apparent when the purified components are studied in isolation.

Useful as the SV40 system is, the demonstration that complete synthesis can occur with T antigen and purified cellular components (*50, 51*) shows that proteins that can be mimicked by T antigen, for example, helicases, may not be readily identified. Cellular helicases have been isolated from other systems, however (*52, 53*), and the interaction of these with proteins known to be present at a eukaryotic replication fork will be viewed with interest.

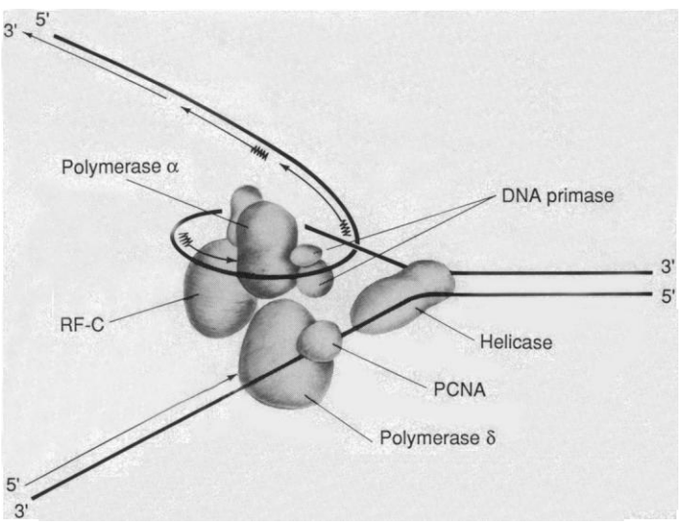

Fig. 2. Schematic representation of a eukaryotic replication fork showing concerted action by DNA polymerase α and δ on opposite sides of the fork. [Reprinted from (*96*) with permission of ICSU Press]

Termination of Replication

Although a paused replication fork can lead to termination at that site (Fig. 1), there is no evidence that specific DNA sequences are required for termination of replication. Instead replication terminates when two forks meet, wherever that may be. On the basis of experiments with replicating SV40, Sundin and Varshavsky (*54*) have proposed that the last few helical turns of DNA between two converging forks might be unwound by wrapping the two progeny DNA duplexes around each other. This would generate a substrate for DNA topoisomerase II, which cuts both strands of a DNA duplex and passes a second duplex through the gap. Support for this model comes from mutations that inactivate DNA topoisomerase II in yeast. These mutants are unable to separate their progeny duplexes but appear blocked in this terminal stage of DNA replication (*55–57*).

A different problem is faced when a replication fork reaches the end of a linear chromosome. This problem is overcome by a

specialized telomere structure that appears to be replicated by a specific enzyme, "telomerase." Telomerase differs from DNA polymerases in that it does not copy the DNA template; instead it adds a simple sequence DNA repeat to the chromosome ends, apparently by copying an RNA template, which is a component of telomerase itself (*58*).

Assembly of Chromatin and Chromosomes

It is not just DNA that must be replicated in S phase, but the entire complex structure of the chromosome, ready for division at mitosis. Assembly of newly replicated DNA into nucleosomes appears to occur only a short way behind the replication fork, with assembly of H3 and H4 preceding the assembly of H2A and H2B (*59, 60*).

Nucleosome assembly mechanisms have been elucidated in two different types of cell-free systems. In *Xenopus* eggs, assembly is mediated by at least two proteins that bind histones and transfer them to DNA (*61–63*). Nucleoplasmin binds and transfers H2A and H2B, and N1 binds and transfers H3 and H4 (*62, 63*). This pathway does not require DNA replication, and it is not clear how abundant these proteins are in other types of cells.

Recently, a protein called CAF-1, which assembles nucleosomes on replicating SV40, was isolated from human cells (*64*). In contrast to assembly in the *Xenopus* egg, nucleosome assembly by CAF-1 occurs only on replicating DNA. It is not obvious that CAF-1 is related to nucleoplasmin or N1, so it will be interesting to see if there are two unrelated mechanisms, or if these two classes of protein are different components of a single, more complex, mechanism. There is evidence that DNA replication accelerates nucleosome assembly in *Xenopus* eggs even though it is not essential (*65*).

There is controversy in the literature over how old nucleosomes segregate at a replication fork. Evidence that preexisting nucleosomes segregate cooperatively to the leading side of a replication fork (*66*) has been challenged twice (*67, 68*), yet a recent origin mapping study (*29*) has obtained results that are difficult to explain unless the original model was correct. Clearly this debate will continue.

In addition to nucleosomes, chromosome scaffolds must also duplicate. How this is achieved may turn out to be a major key to understanding ordered progress through S phase. Scaffold attachment regions of DNA can bind selectively to scaffold proteins in vitro (*69*). Furthermore, ARS elements and centromeres have been shown to be attached to the scaffold in yeast (*70*). It is tempting to speculate that failure to form scaffolds and failure of isolated DNA to bind to scaffolds may contribute to the frustrating lack of initiating mammalian cell-free replication systems and assays for mammalian autonomously replicating sequences.

The Importance of Nuclear Structure

Eukaryotic DNA replication occurs in the complex structural context of the cell nucleus, and there have been repeated suggestions that sites of replication are immobilized on a structural framework, the nuclear "matrix," "cage," or "skeleton" (*71*). It is likely that these structures share elements with the chromosome scaffold, but precise comparisons require a greater knowledge of the key proteins. The initial studies that argued that replication sites were immobilized (*72, 73*) used 2*M* NaCl to remove histones; therefore, they were open to the criticism that high salt concentrations caused aggregation by promoting hydrophobic interactions between soluble molecules. More recent studies have avoided this problem, notably by digesting chromatin out of nuclei that have been encapsulated in agarose beads, performing all steps at physiological ionic strength (*74, 75*). These studies have greatly reinforced the case for believing that sites of replication are immobilized on a structural framework within the nucleus.

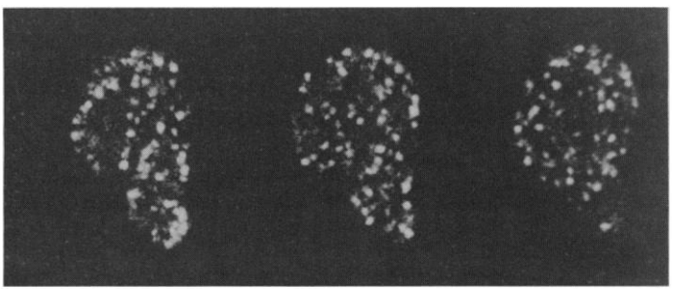

Fig. 3. Discrete sites of DNA replication visualized by pulse labeling with biotinylated dUTP, followed by treatment with fluorescent streptavidin. Shown is a series of confocal optical sections through a *Xenopus* sperm nucleus replicating in a *Xenopus* egg extract in vitro. Each bright spot contains several hundred replication forks that remain clustered throughout S phase. Details are in (*79*) (×1650). [Micrograph by A. D. Mills]

Strong support for the concept of a complex structural framework for eukaryotic DNA replication comes from light microscopy of pulse-labeled nuclei. When replicating nuclei are pulse labeled with either BrdU or biotinylated deoxyuridine 5′-triphosphate (dUTP), sites of replication can be visualized microscopically (*76–79*). Figure 3 shows a series of confocal optical sections through a nucleus that has been pulse labeled with biotin-11-dUTP while replicating in vitro in a *Xenopus* egg extract (*79*). Replication is confined to a few hundred discrete foci, each of which must consist of several hundred replication forks that appear to remain clustered throughout the entire S phase (*79*). Because there is no evidence that chromatin fibers are arranged as hundreds of parallel fibers, it is difficult to escape the conclusion that the surrounding unreplicated DNA must be spooled through fixed sites of replication as suggested in the early studies of the nuclear matrix.

The growing evidence that eukaryotic DNA replication takes place on an immobilized structural framework may explain why it has been so difficult to obtain eukaryotic cell-free systems that initiate replication efficiently in vitro. A conspicuous exception is the system derived from eggs of *X. laevis*, which replicates nuclei or naked DNA (*11–13*). It is possibly significant that these replication systems were developed from an extract that had previously been shown to assemble nuclear envelopes and to alter nuclear structure (*80*). Furthermore, they assemble naked DNA into pseudonuclei (*11, 12, 81*), and there are numerous correlations to suggest that nuclear structure is important or perhaps essential for DNA replication in this system (*11, 12, 82, 83*).

How Are Rounds of DNA Replication Coupled to the Cell Cycle?

Early cell fusion experiments revealed that replicated nuclei cannot be induced to reenter S phase until after they have passed through mitosis (*84*). Similarly, Blumenthal *et al.* (*3*) noted that they never found one replication fork lying within another. In some way eukaryotes distinguish replicated DNA from unreplicated DNA so that rereplication does not occur until the next cell cycle. Although there are exceptions in which genes are amplified (*85, 86*), replication only once between divisions is a hallmark of eukaryotic cells, distinguishing them from prokaryotes.

Xenopus eggs allow this regulatory mechanism to be reconstructed

on naked DNA (*33*). Injected DNA replicates multiple times when the cell cycle clock of the eggs runs freely, but it replicates only once when the cell cycle clock is arrested. Then unreplicated molecules continue to replicate throughout the incubation, but replicated molecules fail to reinitiate. The most striking feature of these experiments is the lack of any detectable DNA sequence requirement for the discriminatory mechanism to act (*33, 87, 88*). Therefore, a simple mechanism that marks used replication origins with an inhibitor would not be sufficient to explain these experiments. On the other hand, experiments by Roberts and Weintraub (*89*) have obtained evidence for a cis-acting negative mechanism of this type that constrains bovine papilloma virus replication to one round per cycle. Experiments with *Xenopus* eggs do not show that there is no cis-acting negative mechanism. They simply show that such a mechanism on its own is not sufficient to explain the results.

An alternative class of model has been proposed that is based on experiments with the cell-free replication system from *Xenopus* eggs (*90*). The model is based on observations that each nucleus in the extract behaves as an integrated and independent unit of replication (*83*). Thus, although each nucleus may replicate at a different time from its neighbors in the same cell-free extract, all the DNA in any one nucleus replicates as a single unit in a short time, suggesting that the nucleus itself is an important unit of replication. Replicated nuclei failed to rereplicate unless treated with maturation promoting factor, which causes their chromosomes to condense and their nuclear envelopes to break down. However, nuclear envelope permeabilization by itself, without chromosome condensation, was found to be sufficient to allow rereplication (*90*). These results can be explained by a simple model in which an essential initiation factor lacks a nuclear migration signal so that it cannot reach the DNA to bind until the nuclear envelope breaks down at mitosis. After it has bound tightly to DNA it would be used once and only once to license DNA replication, and it would be destroyed during replication. No more "licensing factor" could gain access to the DNA to permit rereplication until the nuclear envelope breaks down again during the next mitosis (*90*). At present this remains a hypothetical model that can explain experimental observations, so a direct assay for such a factor would be valuable.

Coordination of DNA Replication with Transcription

As mentioned above, the length of S phase varies in the life cycle of *Drosophila* from less than 4 min in early embryos to about 10 hours in adult cells (*3*). Similarly, *Xenopus* embryos have S phases of only 25 min compared to many hours in adult cells (*91*). In both species there is evidence that the shorter S phases of embryos are largely achieved by initiating replication at closer intervals on DNA (*3, 91*). In addition, there is evidence that early embryos are transcriptionally quiescent during exactly the same cell cycles that show maximal replication rates (*92, 93*). A possibility is that the close spacing of replication forks in early embryos can only be achieved on transcriptionally inactive templates and that the longer spacing of replication forks in transcriptionally active cells is required to coordinate the traffic of RNA polymerases and DNA polymerases on the same DNA template.

This interpretation might explain why several replication origins contain promoter elements, or bind transcription factors, or are close to transcriptional promoters (*10, 94*). In addition it might help to explain why transcriptionally quiescent *Xenopus* eggs are able to replicate injected foreign DNA without regard to its sequence.

A strong argument states that the relative orientation of replication forks and transcription units is fundamentally important for survival of *Escherichia coli* (*95*). RNA polymerases on most highly transcribed genes travel in the same direction as replicating DNA polymerases. Attempts to reverse this relation are not viable. The same problem could be a major factor in determining the positions of the many initiations of replication within eukaryotic chromosomes.

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Dominoes and Clocks: The Union of Two Views of the Cell Cycle

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We review the recent advances in understanding transitions within the cell cycle. These have come from both genetic and biochemical approaches. We discuss the phylogenetic conservation of the mechanisms that induce mitosis and their implications for other transitions in the cell cycle.

THE CELL CYCLE IS THE SET OF EVENTS THAT IS RESPONSIBLE for the duplication of the cell. The recent advances in our understanding of the cell cycle have come from two approaches. Geneticists attempted to understand the cell cycle by analyzing mutations that arrested the cell cycle of somatic cells at specific points, whereas embryologists and physiologists examined natural points of cell cycle arrest and the agents that induced the embryonic cell cycle to proceed.

The genetic approach to the somatic cell cycle evolved from prokaryotic genetics in the 1950s and 1960s. With genetics, researchers successfully explained complicated processes, such as phage morphogenesis, as a linear sequence of events. The most extreme models of these processes suggested that they would resemble metabolic pathways: the initiation of each step in the pathway would be dependent on the completion of the preceding step, because the product of the earlier step was the substrate for the latter one; specific genes were assumed to execute each step. When this approach was applied to yeast, first in the budding yeast by Hartwell and his colleagues (*1*) and later in the fission yeast by Nurse and his colleagues (*2*), the result was a description of the cell cycle as a set of dependent reactions. The basis of this dependency is discussed in the accompanying review by Hartwell and Weinert (*3*). The physiological and embryological approach was championed by researchers who favored marine and amphibian eggs. They argued that eggs and oocytes were the simplest systems for studying the basic processes of the cell cycle, because they were specialized for rapid cell division. The result of their investigations was a description of the cell cycle as a biochemical machine that oscillated between two states, mitosis and interphase, and whose oscillations were independent of the completion of many of the cell cycle events. Initially the two views of the cell cycle, one as a set of dependent reactions (the domino theory) and the other as a biochemical oscillator (the clock theory), seemed incompatible.

The cell fusion experiments of Rao and Johnson (*4*) supported both points of view. The fusion of cells in mitosis with cells in any other state induced some form of mitotic response in the interphase nucleus and supported the embryological model of distinct mitotic and interphase cytoplasmic states, with the mitotic state dominant over all interphase states (*4*). Fusion experiments, however, also supported the idea of a dependent cell cycle, since in any fusion between two interphase cells at different stages of the cell cycle, the advanced nucleus waits for the completion of events in the retarded nucleus before progressing in the cell cycle (*4*).

In this review we discuss recent evidence from both traditions that has led to a unified view of the eukaryotic cell cycle. This synthesis suggests that a single biochemical mechanism underlies the cell cycle in all eukaryotic organisms. We have concentrated on the reactions that regulate progress through the cell cycle and do not discuss the mechanism of individual cell cycle events such as DNA synthesis or nuclear envelope breakdown and reformation.

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