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Alberts B, Johnson A, Lewis J, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002.

The Initiation and Completion of DNA Replication in Chromosomes

We have seen how a set of replication proteins rapidly and accurately generates two daughter DNA double helices behind a moving replication fork. But how is this replication machinery assembled in the first place, and how are replication forks created on a double-stranded DNA molecule? In this section, we discuss how DNA replication is initiated and how cells carefully regulate this process to ensure that it takes place at the proper positions on the chromosome and also at the appropriate time in the life of the cell. We also discuss a few of the special problems that the replication machinery in eucaryotic cells must overcome. These include the need to replicate the enormously long DNA molecules found in eucaryotic chromosomes, as well as the difficulty of copying DNA molecules that are tightly complexed with histones in nucleosomes.

DNA Synthesis Begins at Replication Origins

As discussed previously, the DNA double helix is normally very stable: the two DNA strands are locked together firmly by a large number of hydrogen bonds formed between the bases on each strand. To be used as a template, the double helix must first be opened up and the two strands separated to expose unpaired bases. As we shall see, the process of DNA replication is begun by special *initiator proteins* that bind to double-stranded DNA and pry the two strands apart, breaking the hydrogen bonds between the bases.

The positions at which the DNA helix is first opened are called replication origins (Figure 5-29). In simple cells like those of bacteria or yeast, origins are specified by DNA sequences several hundred nucleotide pairs in length. This DNA contains short sequences that attract initiator proteins, as well as stretches of DNA that are especially easy to open. We saw in Figure 4-4 that an A-T base pair is held together by fewer hydrogen bonds than a G-C base pair. Therefore, DNA rich in A-T base pairs is relatively easy to pull apart, and regions of DNA enriched in A-T pairs are typically found at replication origins.

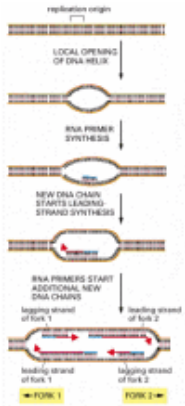


Figure 5-29

A replication bubble formed by replication fork initiation. This diagram outlines the major steps involved in the initiation of replication forks at replication origins. The structure formed at the last step, in which both strands of the parental DNA (more...)

Although the basic process of replication fork initiation, depicted in Figure 5-29 is the same for bacteria and eucaryotes, the detailed way in which this process is performed and regulated differs between these two groups of organisms. We first consider the simpler and better-understood case in bacteria and then turn to the more complex situation found in yeasts, mammals, and other eucaryotes.

Bacterial Chromosomes Have a Single Origin of DNA Replication

The genome of *E. coli* is contained in a single circular DNA molecule of 4.6×10^6 nucleotide pairs. DNA replication begins at a single origin of replication, and the two replication forks assembled there proceed (at approximately 500–1000 nucleotides per second) in opposite directions until they meet up roughly halfway around the chromosome (Figure 5-30). The only point at which *E. coli* can control DNA replication is initiation: once the forks have been assembled at the origin, they move at a relatively constant speed until replication is finished. Therefore, it is not surprising that the initiation of DNA replication is a highly regulated process. It begins when initiator proteins bind in multiple copies to specific sites in the replication origin, wrapping the DNA around the proteins to form a large protein–DNA complex. This complex then binds a DNA helicase and loads it onto an adjacent DNA single strand whose bases have been exposed by the assembly of the initiator protein–DNA complex. The DNA primase joins the helicase, forming the primosome, which moves away from the origin and makes an RNA primer that starts the first DNA chain (Figure 5-31). This quickly leads to the assembly of the remaining proteins to create two replication forks, with protein complexes that move away from the origin in opposite directions. These protein machines continue to synthesize DNA until all of the DNA template downstream of each fork has been replicated.

Figure 5-30

DNA replication of a bacterial genome. It takes *E. coli* about 40 minutes to duplicate its genome of 4.6×10^6 nucleotide pairs. For simplicity, no Okazaki fragments are shown on the lagging strand. What happens as the two replication forks approach (more...)

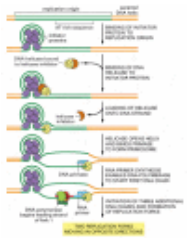


Figure 5-31

The proteins that initiate DNA replication in bacteria. The mechanism shown was established by studies *in vitro* with a mixture of highly purified proteins. For *E. coli* DNA replication, the major initiator protein is the dnaA protein; the primosome is (more...)

In *E. coli,* the interaction of the initiator protein with the replication origin is carefully regulated, with initiation occurring only when sufficient nutrients are available for the bacterium to complete an entire round of replication. Not only is the activity of the initiator protein controlled, but an origin of replication that has just been used experiences a “refractory period,” caused by a delay in the methylation of newly synthesized A nucleotides. Further initiation of replication is blocked until these As are methylated (Figure 5-32).

Figure 5-32

Methylation of the *E. coli* replication origin creates a refractory period for DNA initiation. DNA methylation

occurs at GATC sequences, 11 of which are found in the origin of replication (spanning about 250 nucleotide pairs). About 10 minutes after replication (more...)

Eucaryotic Chromosomes Contain Multiple Origins of Replication

We have seen how two replication forks begin at a single replication origin in bacteria and proceed in opposite directions, moving away from the origin until all of the DNA in the single circular chromosome is replicated. The bacterial genome is sufficiently small for these two replication forks to duplicate the genome in about 40 minutes. Because of the much greater size of most eucaryotic chromosomes, a different strategy is required to allow their replication in a timely manner.

A method for determining the general pattern of eucaryotic chromosome replication was developed in the early 1960s. Human cells growing in culture are labeled for a short time with ^{3}H-thymidine so that the DNA synthesized during this period becomes highly radioactive. The cells are then gently lysed, and the DNA is streaked on the surface of a glass slide coated with a photographic emulsion. Development of the emulsion reveals the pattern of labeled DNA through a technique known as *autoradiography*. The time allotted for radioactive labeling is chosen to allow each replication fork to move several micrometers along the DNA, so that the replicated DNA can be detected in the light microscope as lines of silver grains, even though the DNA molecule itself is too thin to be visible. In this way, both the rate and the direction of replication-fork movement can be determined (Figure 5-33). From the rate at which tracks of replicated DNA increase in length with increasing labeling time, the replication forks are estimated to travel at about 50 nucleotides per second. This is approximately one-tenth of the rate at which bacterial replication forks move, possibly reflecting the increased difficulty of replicating DNA that is packaged tightly in chromatin.

Figure 5-33

The experiments that demonstrated the pattern in which replication forks are formed and move on eucaryotic chromosomes. The new DNA made in human cells in culture was labeled briefly with a pulse of highly radioactive thymidine (^{3}H-thymidine). (A) In (more...)

An average-sized human chromosome contains a single linear DNA molecule of about 150 million nucleotide pairs. To replicate such a DNA molecule from end to end with a single replication fork moving at a rate of 50 nucleotides per second would require $0.02 \times 150 \times 10^{6} = 3.0 \times 10^{6}$ seconds (about 800 hours). As expected, therefore, the autoradiographic experiments just described reveal that many forks are moving simultaneously on each eucaryotic chromosome. Moreover, many forks are found close together in the same DNA region, while other regions of the same chromosome have none.

Further experiments of this type have shown the following: (1) Replication origins tend to be activated in clusters, called *replication units,* of perhaps 20–80 origins. (2) New replication units seem to be activated at different times during the cell cycle until all of the DNA is replicated, a point that we return to below. (3) Within a replication unit, individual origins are spaced at intervals of 30,000–300,000 nucleotide pairs from one another. (4) As in bacteria, replication forks are formed in pairs and create a replication bubble as they move in opposite directions away from a common point of origin, stopping only when they collide head-on with a replication

fork moving in the opposite direction (or when they reach a chromosome end). In this way, many replication forks can operate independently on each chromosome and yet form two complete daughter DNA helices.

In Eucaryotes DNA Replication Takes Place During Only One Part of the Cell Cycle

When growing rapidly, bacteria replicate their DNA continually, and they can begin a new round before the previous one is complete. In contrast, DNA replication in most eucaryotic cells occurs only during a specific part of the cell division cycle, called the *DNA synthesis phase* or S phase (Figure 5-34). In a mammalian cell, the S phase typically lasts for about 8 hours; in simpler eucaryotic cells such as yeasts, the S phase can be as short as 40 minutes. By its end, each chromosome has been replicated to produce two complete copies, which remain joined together at their centromeres until the *M phase* (M for *mitosis),* which soon follows. In Chapter 17, we describe the control system that runs the cell cycle and explain why entry into each phase of the cycle requires the cell to have successfully completed the previous phase.

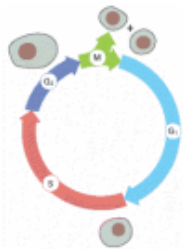


Figure 5-34

The four successive phases of a standard eucaryotic cell cycle. During the G_1, S, and G_2 phases, the cell grows continuously. During M phase growth stops, the nucleus divides, and the cell divides in two. DNA replication is confined to the part of interphase (more...)

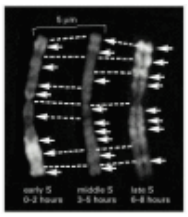
In the following sections, we explore how chromosome replication is coordinated within the S phase of the cell cycle.

Different Regions on the Same Chromosome Replicate at Distinct Times in S Phase

In mammalian cells, the replication of DNA in the region between one replication origin and the next should normally require only about an hour to complete, given the rate at which a replication fork moves and the largest distances measured between the replication origins in a replication unit. Yet S phase usually lasts for about 8 hours in a mammalian cell. This implies that the replication origins are not all activated simultaneously and that the DNA in each replication unit (which, as we noted above, contains a cluster of about 20–80 replication origins) is replicated during only a small part of the total S-phase interval.

Are different replication units activated at random, or are different regions of the genome replicated in a specified order? One way to answer this question is to use the thymidine analogue bromodeoxyuridine (BrdU) to label the newly synthesized DNA in synchronized cell populations, adding it for different short periods throughout S phase. Later, during M phase, those regions of the mitotic chromosomes that have incorporated BrdU into their DNA can be recognized by their altered staining properties or by means of anti-BrdU antibodies. The results show that different regions of each chromosome are replicated in a reproducible order during S phase (Figure 5-35). Moreover, as one would expect from the clusters of replication forks seen in DNA autoradiographs (see Figure 5-33), the timing of replication is coordinated over large regions of the chromosome.

Figure 5-35



Different regions of a chromosome are replicated at different t
These light micrographs show stained mitotic chromosomes ir
replicating DNA has been differentially labeled during differe
intervals of the preceding S (more...)

Highly Condensed Chromatin Replicates Late, While Genes in Less Condensed Chromatin Tend to Replicate Early

It seems that the order in which replication origins are activated depends, in part, on the chromatin structure in which the origins reside. We saw in Chapter 4 that heterochromatin is a particularly condensed state of chromatin, while transcriptionally active chromatin has a less condensed conformation that is apparently required to allow RNA synthesis. Heterochromatin tends to be replicated very late in S phase, suggesting that the timing of replication is related to the packing of the DNA in chromatin. This suggestion is supported by an examination of the two X chromosomes in a female mammalian cell. While these two chromosomes contain essentially the same DNA sequences, one is active for DNA transcription and the other is not (discussed in Chapter 7). Nearly all of the inactive X chromosome is condensed into heterochromatin, and its DNA replicates late in S phase. Its active homologue is less condensed and replicates throughout S phase.

These findings suggest that those regions of the genome whose chromatin is least condensed, and therefore most accessible to the replication machinery, are replicated first. Autoradiography shows that replication forks move at comparable rates throughout S phase, so that the extent of chromosome condensation seems to influence the time at which replication forks are initiated, rather than their speed once formed.

The above relationship between chromatin structure and the timing of DNA replication is also supported by studies in which the replication times of specific genes are measured. The results show that so-called “housekeeping” genes, which are those active in all cells, replicate very early in S phase in all cells tested. In contrast, genes that are active in only a few cell types generally replicate early in the cells in which the genes are active, and later in other types of cell.

The relationship between chromatin structure and the timing of replication has been tested directly in the yeast *S. cerevisiae*. In one case, an origin that functioned late in S phase, and was found in a transcriptionally silent region of a yeast chromosome, was experimentally relocated to a transcriptionally active region. After the relocation, the origin functioned early in the S phase, indicating that the time in S phase when this origin is used is determined by the origin's location in the chromosome. However, studies with additional yeast origins have revealed the existence of other origins that initiate replication late, even when present in normal chromatin. Thus, the time at which an origin is used can be determined both by its chromatin structure and by its DNA sequence.

Well-defined DNA Sequences Serve as Replication Origins in a Simple Eucaryote, the Budding Yeast

Having seen that a eucaryotic chromosome is replicated using many origins of replication, each of which “fires” at a characteristic time in S phase of the cell cycle, we turn to the nature of these origins of replication. We saw earlier in this chapter that replication origins have been precisely defined in bacteria as specific DNA sequences that allow the DNA replication machinery to assemble on the DNA double helix, form a replication bubble, and move in opposite directions to

produce replication forks. By analogy, one would expect the replication origins in eucaryotic chromosomes to be specific DNA sequences too.

The search for replication origins in the chromosomes of eucaryotic cells has been most productive in the budding yeast *S. cerevisiae.* Powerful selection methods to find them have been devised that make use of mutant yeast cells defective for an essential gene. These cells can survive in a selective medium only if they are provided with DNA that carries a functional copy of the missing gene. If a circular bacterial plasmid with this gene is introduced into the mutant yeast cells directly, it will not be able to replicate because it lacks a functional origin. If random pieces of yeast DNA are inserted into this plasmid, however, only those few plasmid DNA molecules that contain a yeast replication origin can replicate. The yeast cells that carry such plasmids are able to proliferate because they have been provided with the essential gene in a form that can be replicated and passed on to progeny cells (Figure 5-36). A DNA sequence identified by its presence in a plasmid isolated from these surviving yeast cells is called an *autonomously replicating sequence (ARS)*. Most ARSs have been shown to be authentic chromosomal origins of replication, thereby validating the strategy used to obtain them.

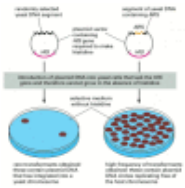


Figure 5-36

The strategy used to identify replication origins in yeast cells. Each of the yeast DNA sequences identified in this way was called an autonomously replicating sequence (ARS), since it enables a plasmid that contains it to replicate in the host cell without (more...)

For budding yeast, the location of every origin of replication on each chromosome can be determined (Figure 5-37). The particular chromosome shown—chromosome III from the yeast *S. cerevisiae*—is less than 1/100 the length of a typical human chromosome. Its origins are spaced an average of 30,000 nucleotides apart; this density of origins should permit a yeast chromosome to be replicated in about 8 minutes.

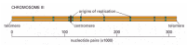


Figure 5-37

The origins of DNA replication on chromosome III of the yeast *S. cerevisiae*. This chromosome, one of the smallest eucaryotic chromosomes known, carries a total of 180 genes. As indicated, it contains nine replication origins.

Genetic experiments in *S. cerevisiae* have tested the effect of deleting various sets of the replication origins on chromosome III. Removing a few origins has little effect, because replication forks that begin at neighboring origins of replication can continue into the regions that lack their own origins. However, as more replication origins are deleted from this chromosome, the chromosome is gradually lost as the cells divide, presumably because it is replicated too slowly.

A Large Multisubunit Complex Binds to Eucaryotic Origins of Replication

The minimal DNA sequence required for directing DNA replication initiation in the yeast *S. cerevisiae* has been determined by performing the experiment shown in Figure 5-36 with smaller and smaller DNA fragments. Each of the yeast replication origins contains a binding site for a

large, multisubunit initiator protein called **ORC**, for origin recognition complex, and several auxiliary binding sites for proteins that help attract ORC to the origin DNA (Figure 5-38).

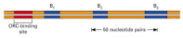


Figure 5-38

An origin of replication in yeast. Comprising about 150 nucleotide pairs, this yeast origin (identified by the procedure shown in Figure 5-36) has a binding site for ORC, a complex of proteins that binds to every origin of replication. The origin depicted (more...)

As we have seen, DNA replication in eucaryotes occurs only in the S phase. How is this DNA replication triggered, and how does the mechanism ensure that a replication origin is used only once during each cell cycle?

As we discuss in Chapter 17, the general answers to these two questions are now known. In brief, the ORC-origin interaction is a stable one that serves to mark a replication origin throughout the entire cell cycle. A prereplicative protein complex is assembled on each ORC during G_1 phase, containing both a hexameric DNA helicase and a helicase loading factor (the Mcm and Cdc6 proteins, respectively). S phase is triggered when a protein kinase is activated that assembles the rest of the replication machinery, allowing an Mcm helicase to start moving with each of the two replication forks that form at each origin. Simultaneously, the protein kinase that triggers S phase prevents all further assembly of the Mcm protein into prereplicative complexes, until this kinase is inactivated at the next M phase to reset the entire cycle (for details, see Figure 17-22).

The Mammalian DNA Sequences That Specify the Initiation of Replication Have Been Difficult to Identify

Compared with the situation in budding yeasts, DNA sequences that specify replication origins in other eucaryotes have been more difficult to define. In humans, for example, the DNA sequences that are required for proper origin function can extend over very large distances along the DNA.

Recently, however, it has been possible to identify specific human DNA sequences, each several thousand nucleotide pairs in length, that serve as replication origins. These origins continue to function when moved to a different chromosomal region by recombinant DNA methods, as long as they are placed in a region where the chromatin is relatively uncondensed. One of these origins is the sequence from the β-globin gene cluster. At its normal position in the genome, the function of this origin depends critically upon distant DNA sequences (Figure 5-39). As discussed in Chapter 7, this distant DNA is known to have a decondensing effect on the chromatin structure that surrounds the origin and includes the β-globin gene; the more open chromatin conformation that results is apparently required for this origin to function, as well as for the β-globin gene to be expressed.

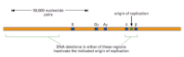


Figure 5-39

Deletions that inactivate an origin of replication in humans. These two deletions are found separately in two individuals who suffer from *thalassemia,* a disorder caused by the failure to express one or more of the genes in the β-globin gene cluster (more...)

We know now that a human ORC complex homologous to that in yeast cells is required for replication initiation and that the human Cdc6 and Mcm proteins likewise have central roles in the initiation process. It therefore seems likely that the yeast and human initiation mechanisms will turn out to be very similar. However, the binding sites for the ORC protein seem to be less specific in humans than they are in yeast, which may explain why the replication origins of humans are longer and less sharply defined than those of yeast.

New Nucleosomes Are Assembled Behind the Replication Fork

In this and the following section we consider several additional aspects of DNA replication that are specific to eucaryotes. As discussed in Chapter 4, eucaryotic chromosomes are composed of the mixture of DNA and protein known as chromatin. Chromosome duplication therefore requires not only that the DNA be replicated but also that new chromosomal proteins be assembled onto the DNA behind each replication fork. Although we are far from understanding this process in detail, we are beginning to learn how the *nucleosome,* the fundamental unit of chromatin packaging, is duplicated. A large amount of new histone protein, approximately equal in mass to the newly synthesized DNA, is required to make the new nucleosomes in each cell cycle. For this reason, most eucaryotic organisms possess multiple copies of the gene for each histone. Vertebrate cells, for example, have about 20 repeated gene sets, most sets containing the genes that encode all five histones (H1, H2A, H2B, H3, and H4).

Unlike most proteins, which are made continuously throughout interphase, histones are synthesized mainly in S phase, when the level of histone mRNA increases about fiftyfold as a result of both increased transcription and decreased mRNA degradation. By a mechanism that depends on special properties of their 3′ ends (discussed in Chapter 7), the major histone mRNAs become highly unstable and are degraded within minutes when DNA synthesis stops at the end of S phase (or when inhibitors are added to stop DNA synthesis prematurely). In contrast, the histone proteins themselves are remarkably stable and may survive for the entire life of a cell. The tight linkage between DNA synthesis and histone synthesis presumably depends on a feedback mechanism that monitors the level of free histone to ensure that the amount of histone made exactly matches the amount of new DNA synthesized.

As a replication fork advances, it must somehow pass through the parental nucleosomes. *In vitro* studies show that the replication apparatus has a poorly understood intrinsic ability to pass through parental nucleosomes without displacing them from the DNA. The chromatin-remodeling proteins discussed in Chapter 4, which destabilize the DNA–histone interface, likely facilitate this process in the cell.

Both of the newly synthesized DNA helices behind a replication fork inherit old histones (Figure 5-40). But since the amount of DNA has doubled, an equal amount of new histones is also needed to complete the packaging of DNA into chromatin. The addition of new histones to the newly synthesized DNA is aided by *chromatin assembly factors (CAFs)*, which are proteins that associate with replication forks and package the newly synthesized DNA as soon as it emerges from the replication machinery. The newly synthesized H3 and H4 histones are rapidly acetylated on their N-terminal tails (discussed in Chapter 4); after they have been incorporated into chromatin, these acetyl groups are removed enzymatically from the histones (Figure 5-41).

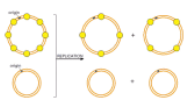


Figure 5-40

A demonstration that histones remain associated with DNA after the replication fork passes. In this experiment, performed *in vitro,* a mixture of two different-sized circular

molecules of DNA (only one of which is assembled into nucleosomes) are replicated (more...)

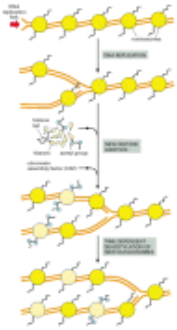


Figure 5-41

The addition of new histones to DNA behind a replication fork. The new nucleosomes are those colored *light yellow* in this diagram; as indicated, some of the histones that form them initially have specifically acetylated lysine side chains (see Figure (more...)

Telomerase Replicates the Ends of Chromosomes

We saw earlier that, because DNA polymerases polymerize DNA only in the 5′-to-3′ direction, synthesis of the lagging strand at a replication fork must occur discontinuously through a backstitching mechanism that produces short DNA fragments. This mechanism encounters a special problem when the replication fork reaches an end of a linear chromosome: there is no place to produce the RNA primer needed to start the last Okazaki fragment at the very tip of a linear DNA molecule.

Bacteria solve this “end-replication” problem by having circular DNA molecules as chromosomes (see Figure 5-30). Eucaryotes solve it in an ingenious way: they have special nucleotide sequences at the ends of their chromosomes, which are incorporated into *telomeres* (discussed in Chapter 4), and attract an enzyme called telomerase. Telomere DNA sequences are similar in organisms as diverse as protozoa, fungi, plants, and mammals. They consist of many tandem repeats of a short sequence that contains a block of neighboring G nucleotides. In humans, this sequence is GGGTTA, extending for about 10,000 nucleotides.

Telomerase recognizes the tip of a G-rich strand of an existing telomere DNA repeat sequence and elongates it in the 5′-to-3′ direction. The telomerase synthesizes a new copy of the repeat, using an RNA template that is a component of the enzyme itself. The telomerase enzyme otherwise resembles other *reverse transcriptases*, enzymes that synthesize DNA using an RNA template (Figure 5-42). The enzyme thus contains all the information used to maintain the characteristic telomere sequences. After several rounds of extension of the parental DNA strand by telomerase, replication of the lagging strand at the chromosome end can be completed by using these extensions as a template for synthesis of the complementary strand by a DNA polymerase molecule (Figure 5-43).

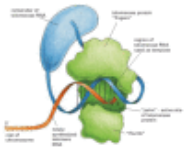


Figure 5-42

The structure of telomerase. The telomerase is a protein–RNA complex that carries an RNA template for synthesizing a repeating, G-rich telomere DNA sequence. Only the part of the telomerase protein homologous to reverse transcriptase is shown (more...)

Figure 5-43

Telomere replication. Shown here are the reactions involved in synthesizing the repeating G-rich sequences that form the ends of the chromosomes (telomeres) of diverse eucaryotic

organisms. The 3′ end of the parental DNA strand is extended by (more...)

The mechanism just described ensures that the 3′ DNA end at each telo-mere is always slightly longer than the 5′ end with which it is paired, leaving a protruding single-stranded end (see Figure 5-43). Aided by specialized proteins, this protruding end has been shown to loop back to tuck its single-stranded terminus into the duplex DNA of the telomeric repeat sequence (Figure 5-44). Thus, the normal end of a chromosome has a unique structure, which protects it from degradative enzymes and clearly distinguishes it from the ends of the broken DNA molecules that the cell rapidly repairs (see Figure 5-53).

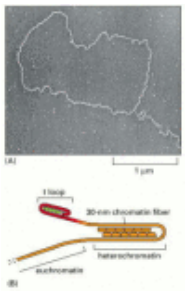


Figure 5-44

The t-loops at the end of mammalian chromosomes. (A) Electron micrograph of the DNA at the end of an interphase human chromosome. The chromosome was fixed, deproteinated, and artificially thickened before viewing. The loop seen here is approximately 15,000 (more...)

Telomere Length Is Regulated by Cells and Organisms

Because the processes that grow and shrink each telomere sequence are only approximately balanced, a chromosome end contains a variable number of telomeric repeats. Not surprisingly, experiments show that cells that proliferate indefinitely (such as yeast cells) have homeostatic mechanisms that maintain the number of these repeats within a limited range (Figure 5-45).

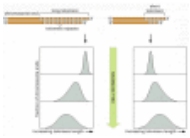


Figure 5-45

A demonstration that yeast cells control the length of their telomeres. In this experiment, the telomere at one end of a particular chromosome is artificially made either longer *(left)* or shorter *(right)* than average. After many cell divisions, the chromosome (more...)

In the somatic cells of humans, the telomere repeats have been proposed to provide each cell with a counting mechanism that helps prevent the unlimited proliferation of wayward cells in adult tissues. According to this idea, our somatic cells are born with a full complement of telomeric repeats; however, the telomerase enzyme is turned off in a tissue like the skin, so that each time a cell divides, it loses 50–100 nucleotides from each of its telomeres. After many cell generations, the descendent cells will inherit defective chromosomes (because their tips cannot be replicated completely) and consequently will withdraw permanently from the cell cycle and cease dividing—a process called *replicative cell senescence* (discussed in Chapter 17). In theory, such a mechanism could provide a safeguard against the uncontrolled cell proliferation of abnormal cells in somatic tissues, thereby helping to protect us from cancer.

The idea that telomere length acts as a “measuring stick” to count cell divisions and thereby regulate the cell's lifetime has been tested in several ways. For certain types of human cells grown in tissue culture, the experimental results support such a theory. Human fibroblasts normally proliferate for about 60 cell divisions in culture before undergoing replicative senescence. Like most other somatic cells in humans, fibroblasts fail to produce telomerase, and their telomeres gradually shorten each time they divide. When telomerase is provided to the

fibroblasts by inserting an active telomerase gene, telomere length is maintained and many of the cells now continue to proliferate indefinitely. It therefore seems clear that telomere shortening can count cell divisions and trigger replicative senescence in human cells.

It has been proposed that this type of control on cell proliferation is important for the maintenance of tissue architecture and that it is also somehow responsible for the aging of animals like ourselves. These ideas have been tested by producing transgenic mice that lack telomerase. The telomeres in mouse chromosomes are about five times longer than human telomeres, and the mice must therefore be bred through three or more generations before their telomeres have shrunk to the normal human length. It is therefore perhaps not surprising that the mice initially develop normally. More importantly, the mice in later generations develop progressively more defects in some of their highly proliferative tissues. But these mice do not seem to age prematurely overall, and the older animals have a pronounced tendency to develop tumors. In these and other respects these mice resemble humans with the genetic disease *dyskeratosis congenita,* which has also been attributed to premature telomere shortening. Individuals afflicted with this disease show abnormalities in various epidermal structures (including skin, nails, and tear ducts) and in the production of red blood cells.

It is clear from the above observations that controlling cell proliferation by the removal of telomeres poses a risk to an organism, because not all of the cells that lack functional telomeres in a tissue will stop dividing. Others apparently become genetically unstable, but continue to divide giving rise to variant cells that can lead to cancer. Thus, one can question whether the observed absence of telomerase from most human somatic cells provides an evolutionary advantage, as suggested by those who postulate that telomere shortening tends to protect us from cancer and other proliferative diseases.

Summary

The proteins that initiate DNA replication bind to DNA sequences at a replication origin to catalyze the formation of a replication bubble with two outward-moving replication forks. The process begins when an initiator protein–DNA complex is formed that subsequently loads a DNA helicase onto the DNA template. Other proteins are then added to form the multienzyme "replication machine" that catalyzes DNA synthesis at each replication fork.

In bacteria and some simple eucaryotes, replication origins are specified by specific DNA sequences that are only several hundred nucleotide pairs long. In other eucaryotes, such as humans, the sequences needed to specify an origin of DNA replication seem to be less well defined, and the origin can span several thousand nucleotide pairs.

Bacteria typically have a single origin of replication in a circular chromosome. With fork speeds of up to 1000 nucleotides per second, they can replicate their genome in less than an hour. Eucaryotic DNA replication takes place in only one part of the cell cycle, the S phase. The replication fork in eucaryotes moves about 10 times more slowly than the bacterial replication fork, and the much longer eucaryotic chromosomes each require many replication origins to complete their replication in a typical 8-hour S phase. The different replication origins in these eucaryotic chromosomes are activated in a sequence, determined in part by the structure of the chromatin, with the most condensed regions of chromatin beginning their replication last. After the replication fork has passed, chromatin structure is re-formed by the addition of new histones to the old histones that are directly inherited as nucleosomes by each daughter DNA molecule.

Eucaryotes solve the problem of replicating the ends of their linear chromosomes by a specialized end structure, the telomere, which requires a special enzyme, telomerase. Telomerase extends the telomere DNA by using an RNA template that is an integral part of the enzyme itself,

producing a highly repeated DNA sequence that typically extends for 10,000 nucleotide pairs or more at each chromosome end.

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