Lecture -2-

The Microbial Genetics

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Replication of DNA

A DNA strand can act as a template for synthesis of a new nucleic acid strand in which each base forms a hydrogen-bonded pair with one on the template strand (G with C, A with T, or A with U for RNA molecules). The new sequence is thus complementary to the template strand. The copying of DNA molecules to produce more DNA is known as replication; the synthesis of RNA using a DNA template is called transcription. Replication is a much more complicated process than implied by the above statement. Some of the main features are summarized in Figure 1.9.

The **opposite polarity of the DNA strands** is a complicating factor. One of the new strands (the 'leading' strand) can be synthesized continuously in the 5′ to 3′ direction. The enzyme responsible for this synthesis is **DNA polymerase III**. With the other new strand however, the overall effect is of growth in the 5′ to 3′ direction. Since nucleic acids can only be synthesized in the 5′ to 3′ direction, the new 3′ to 5′strand (the 'lagging' strand) has to be made in short fragments (known as Okazaki fragments) which are subsequently joined together by the action of another enzyme, DNA ligase.

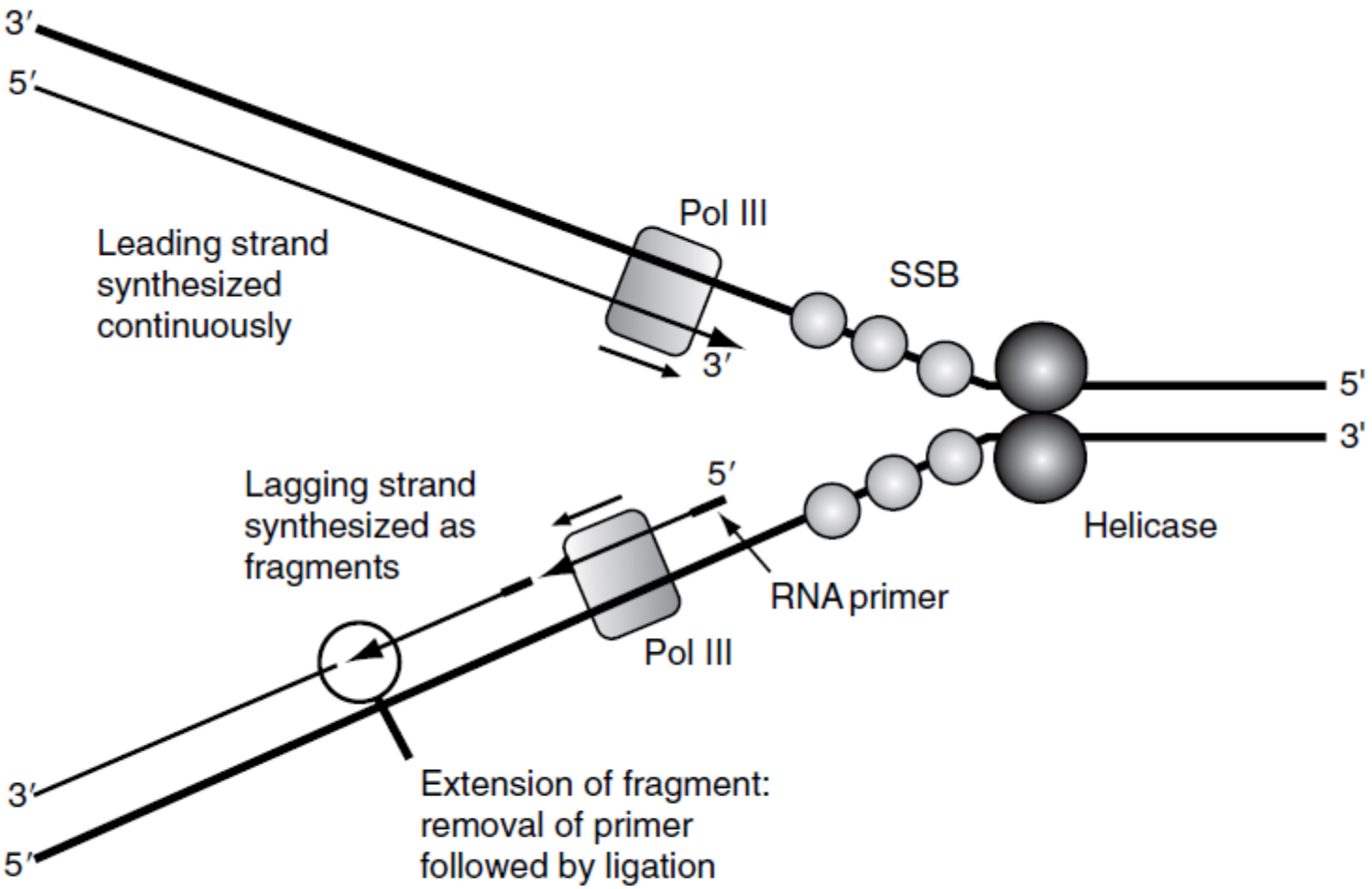


Figure 1.9 Simplified view of the main features of DNA replication. Note that the diagram does not show the helical structure of the DNA

Furthermore, DNA polymerases are incapable of starting a new DNA strand, but can only extend a previously existing molecule. This restriction does not apply to RNA polymerases, which are able to initiate synthesis of new nucleic acids.

Each fragment is therefore started with a short piece of RNA, produced by the action of a special **RNA polymerase (primase).** This **RNA primer** can then be extended by **DNA polymerase III**. The **primer is subsequently removed, and the gap filled in, by a different DNA polymerase (DNA polymerase I**); this enzyme can carry out both of these actions since it has exonuclease as well as polymerase activity. After the gap has been filled, the fragments that have been produced are joined together by DNA ligase.

Unwinding and rewinding

Before any of these events can take place, it is necessary for the two strands to be separated, for a short region at least. This is achieved by enzymes known as **helicases** which bind to the template strand and move along it, separating the two strands.

The separated strands are prevented from re-associating by the binding of another protein, **the single-stranded DNA binding protein or SSB**. A number of copies of the SSB will bind to the DNA strands, maintaining a region of DNA in an extended single-stranded form.

The twisting of the two DNA strands around each other. DNA molecules within the cell cannot normally rotate freely. In bacterial cells for example the DNA is usually circular. Therefore it is not possible to produce a pair of daughter molecules by just separating the two strands and synthesizing the complementary strands.

The strands have to be unwound to be separated. If they are not free to rotate, separating the strands at one point will cause overwinding further along. Unless this problem is overcome, the molecules would quickly become hopelessly tangled. (This can be demonstrated with the use of lengths of string!). The resolution of the problem requires **the action of topoisomerases.** By allowing the double helix to unwind ahead of the replication fork, they permit the strands to separate for replication. One topoisomerase, **DNA gyrase,** has the important role of introducing negative supercoils into the newly replicated DNA.

Fidelity of Replication: proof-reading

It is essential that the newly synthesized DNA is a precise (complementary) copy of the template strand. This does not arise simply by the nucleotides aligning themselves in the right position, but involves the specificity of the DNA polymerase in selecting nucleotides that are correctly aligned.

Most DNA polymerases are more complex enzymes than the name suggests, as they also possess exonuclease activity. We have already encountered one such activity: the removal of the RNA primer from the Okazaki fragments is achieved by means of the 5′ to 3′ exonuclease activity of the DNA polymerase (i.e. it can remove bases from the 5’ end of a chain) as it extends the following fragment.

The fidelity of replication is enhanced by a second exonuclease function of DNA polymerases: the 3′ to 5′ exonuclease activity, which is able to remove the nucleotide at the growing end (3′ end) of the DNA chain. This is not as perverse as it sounds, since the 3′ to 5′ exonuclease only operates if there is an incorrectly paired base at the 3′ end. The DNA polymerase will only extend the DNA chain, by adding nucleotides to the 3′end, if the last base at the 30 end is correctly paired with the template strand. If it is not, polymerization will stop, and the 3′ to 5′ exonuclease function will remove the incorrect nucleotide, allowing a further attempt to be made. The reasons for the occurrence of errors in adding bases to the growing DNA chain .

This mechanism of correcting errors, known as proof-reading or error checking, adds considerably to the fidelity of replication, thus reducing the rate of spontaneous mutation. There is a price to be paid however, as extensive error checking will slow down the rate of replication. The balance between the rate of replication and the extent of error-checking will be determined by the nature of the DNA polymerase itself. Some DNA polymerases do not show efficient proofreading and therefore result in a much higher degree of spontaneous errors. **The rate of spontaneous mutation shown by an organism is therefore (at least in part) a genetic characteristic that is subject to evolutionary pressure.**

The fidelity of replication is further enhanced by DNA repair mechanisms which are described later in this chapter.

Chromosome replication and cell division

Bacterial cells are generally regarded as having a single, circular chromosome. This is a simplification in several ways. **Firstly**, many bacteria often contain additional DNA molecules known as plasmids. In most cases these are additional and dispensable elements, but in some bacterial species all strains carry two or more different DNA molecules, both (or all) of which appear to be essential for normal growth. These can equally well be regarded as essential plasmids or as additional chromosomes. **Secondly**, not all bacterial DNA is circular. Some bacteria (notably Streptomyces) have a linear chromosome and/ or linear plasmids.

More fundamentally, immediately before cell division there must be at least two complete copies of the chromosome, in order to ensure that both daughter cells acquire a copy. Therefore, the chromosome must replicate in tune with the cell division cycle, which means that at an intermediate time in the cycle part of the chromosome will have been copied, with the consequence that there are at least two copies of this part of the chromosome.

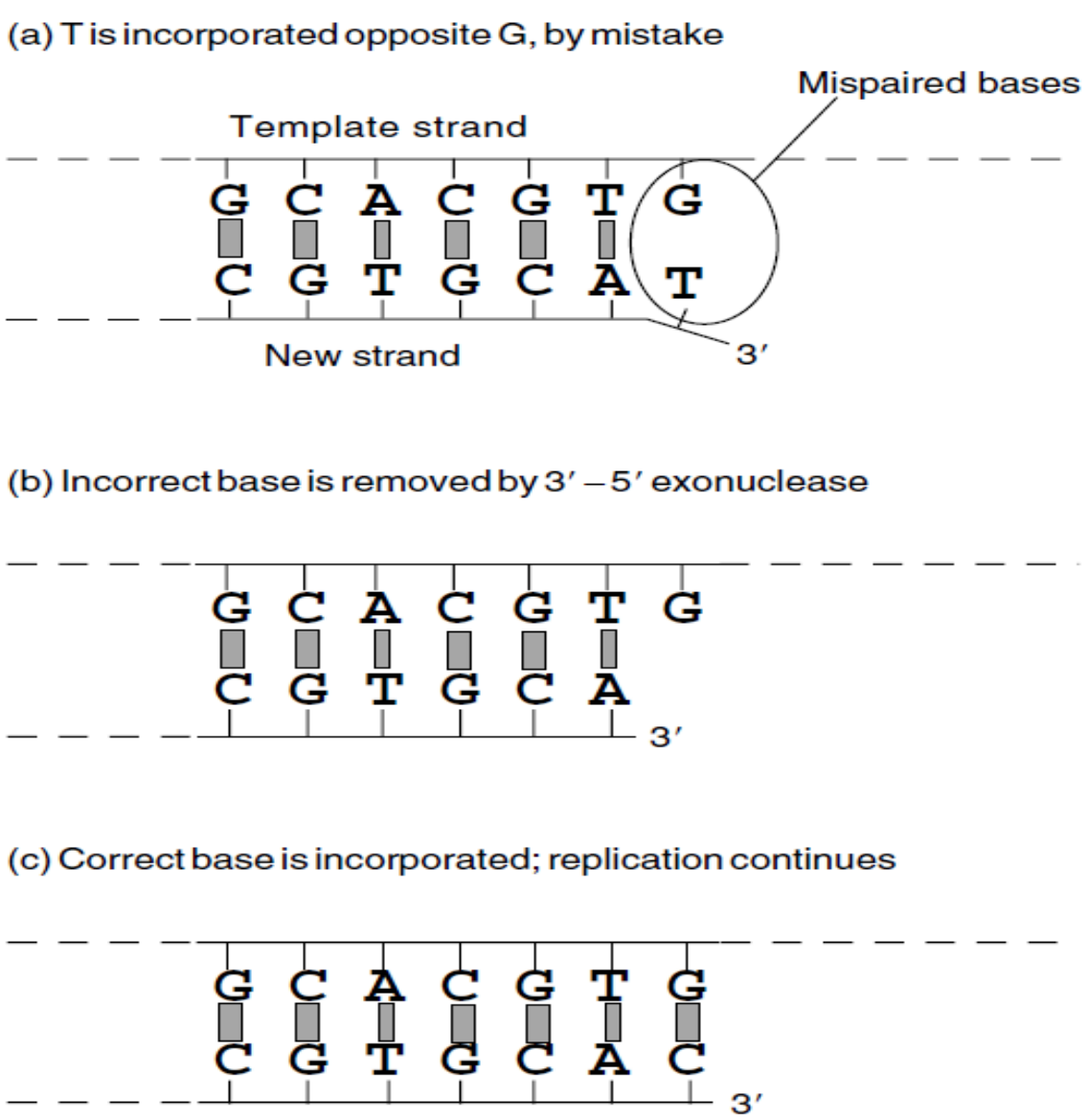
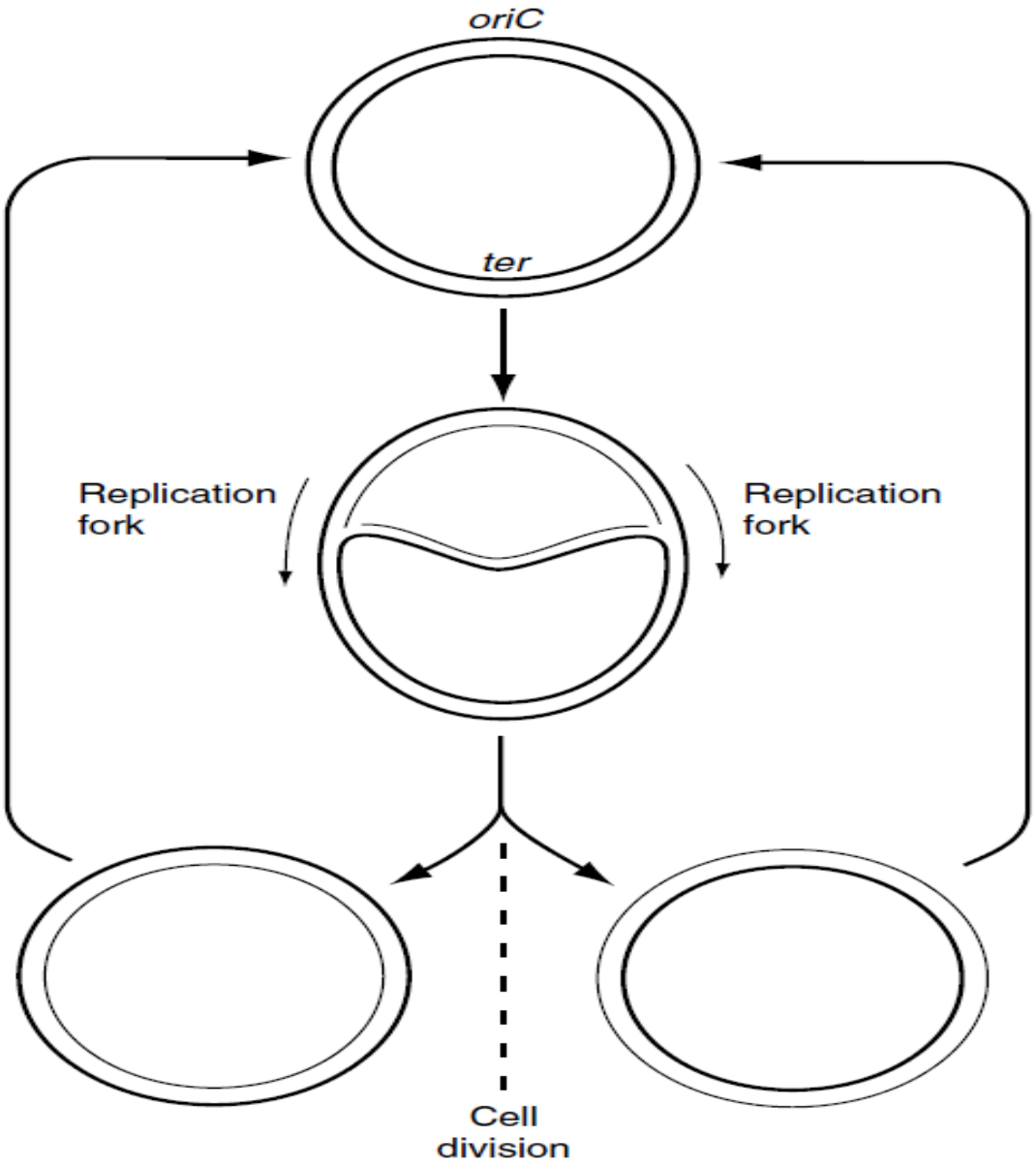


Figure 1.10 Elimination of mispaired bases by proof-reading. (a) An incorrect base has been added to the growing DNA strand; this will prevent further extension. (b) The mispaired base is removed by the 3′—5′ exonuclease action of DNA polymerase. (c) The correct base is added to the 3′ end; DNA synthesis continues

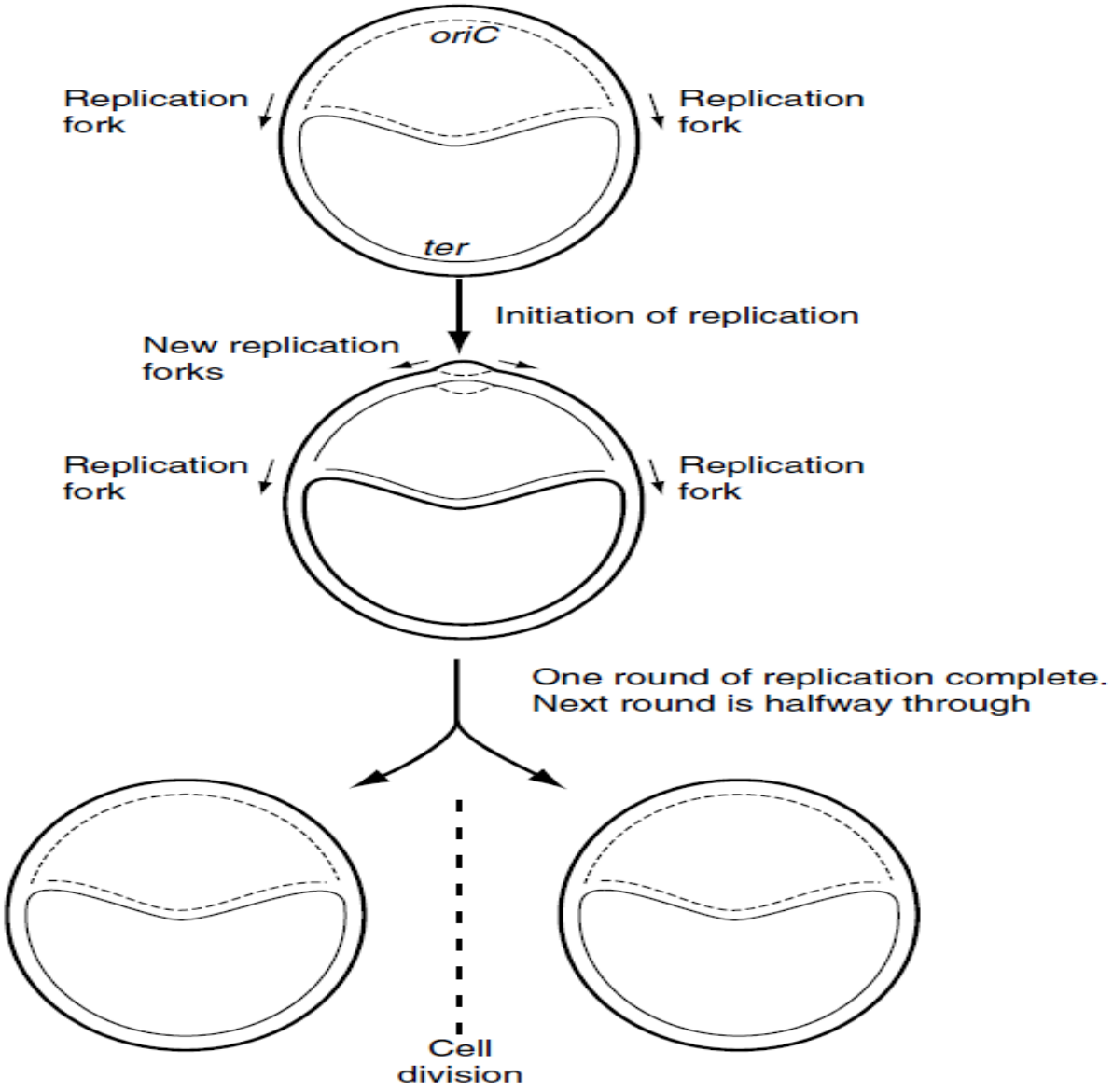
Replication of a bacterial chromosome normally starts at a fixed point (the **origin of replication**, oriV) and proceeds in both directions to a **termination point** (ter) that is approximately opposite to the origin.

Replication of a bacterial chromosome in E. coli, this takes about 40 min, there is then a period of about 20 min before cell division, making a **total of 60 min between the initiation of replication and cell division**. If the growth rate of the cells is changed by using a richer or poorer medium, this time remains much the same. However under favorable conditions, E. coli will grow much faster than that – dividing perhaps every 20 min.

How can the cells be dividing faster than the chromosome replicates and still allow every daughter cell to acquire a complete copy of the chromosome? The answer lies in the timing of the initiation of replication. Initiation is stimulated, not by cell division, but as a function of the size of the cell. Consequently, when the cells are growing rapidly, there are several sets of replication forks copying the chromosome – so that when the cell is ready to divide there are not just a pair of completely replicated chromosomes, but each of these has in turn already been partly replicated by second pair of replication forks.



gure 1.11 Chromosome replication. Bi-directional replication starts at *oriC* and ntinues to the termination site *ter*, producing two double-stranded molecules



gure 1.12 Chromosome replication at higher growth rates. When the interval tween cell divisions is less than the time needed for replication of the chromosome, a w round of replication starts before the previous one has finished

There are two key regulatory points to be considered:

the link between the completion of chromosome replication and subsequent cell division, and the control of the initiation of replication.

Both are too complex (and still incompletely understood) to be considered fully here, but we can consider some aspects.

On the **first point**, one (simplified) model is that the replicating chromosome occupies a region of the membrane at the midpoint of the cell which prevents the initiation of cell division at that point. When replication has finished, the two separate molecules can be pulled apart, towards the poles of the cell, thus freeing the site for cell division to start.

The second point, the control of initiation. Initiation of new rounds of replication is triggered when the cell reaches a critical mass. It is tempting to think that this means that an inhibitor of replication is diluted out as the cell grows, but it is far from that simple. However, we know that initiation requires a protein called **DnaA** which binds to specific DNA sequences known as DnaA boxes; the origin of replication contains a number of DnaA boxes. Wrapping the DNA around the aggregated DnaA proteins facilitates the separation of the strands that is necessary for the initiation of replication. However, the full story of the control of initiation is more complex and still incompletely understood.

One feature worth noting is that **each replication origin fires only once at the time of initiation**. Mature DNA is methylated by addition of methyl groups to adenine residues at certain positions. However, the newly synthesized DNA strand is not methylated and so the double-stranded DNA contains one methylated strand (the old strand) and one non-methylated one (the new strand). This **hemimethylated DNA** is refractory to the initiation of replication, and thus the newly replicated origin will not be available for another initiation event until the second strand has been methylated.

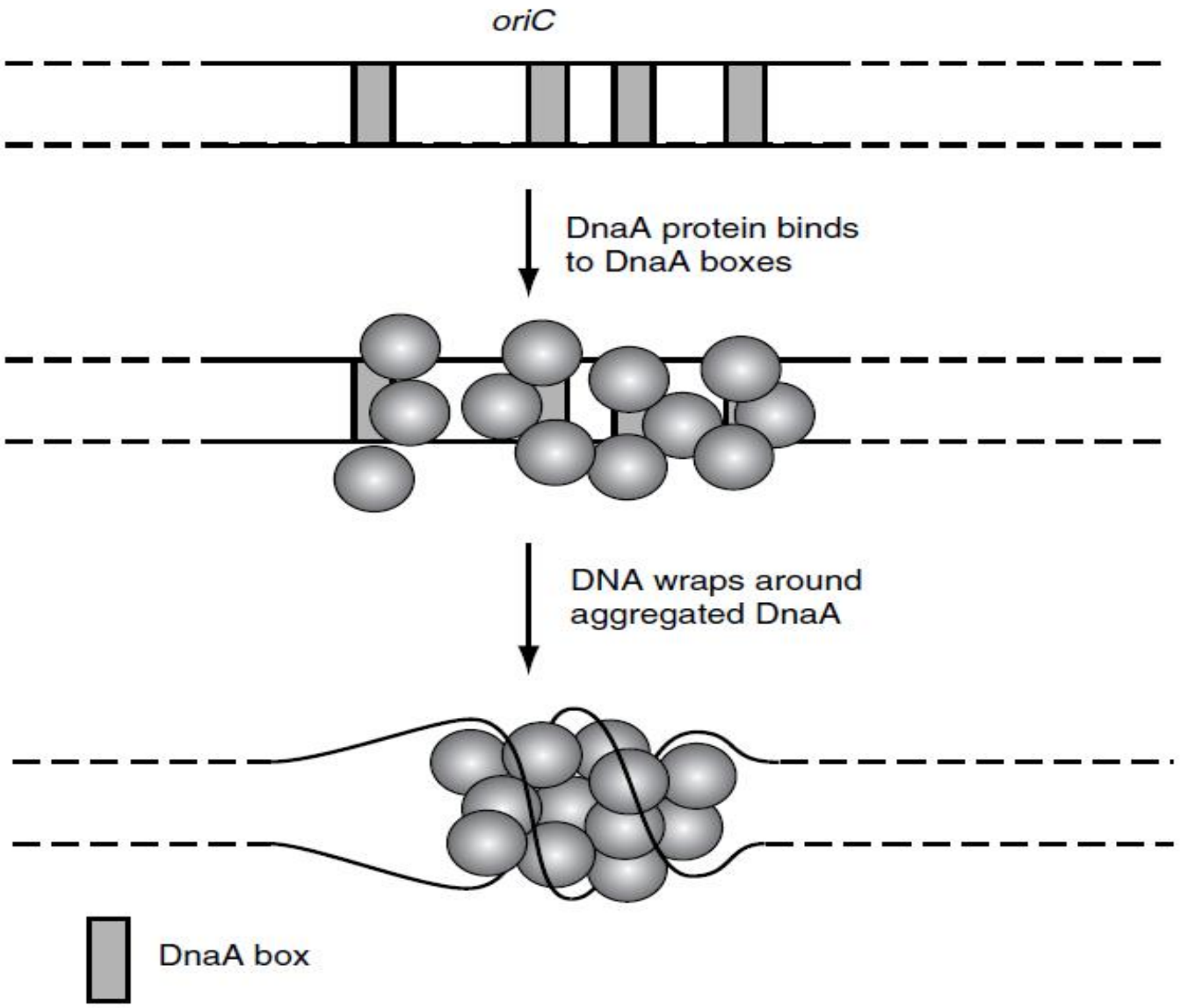


Figure 1.13 Binding of DnaA protein to DnaA boxes at *oriC*. The DnaA protein binds to several sites (DnaA boxes) at the origin of replication; wrapping the DNA around the bound DnaA helps to separate the strands, enabling the initiation of replication

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For example, in E. coli, the DNA polymerase III holoenzyme synthesizes DNA at approximately 750 nucleotides per second, and can extend a DNA strand for several thousand nucleotides without dissociating from the template.