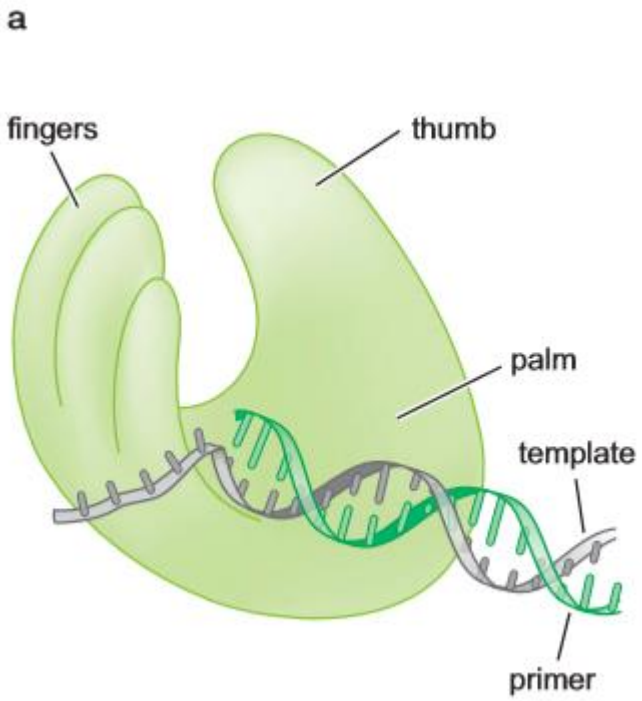

This bend serves to expose only the first template base after the primer at the catalytic site and avoids any confusion concerning which template base should pair with then ext nucleotide to be added (Fig.9-8). In contrast to the fingers and the palm,

The thumb domain is not intimately involved in catalysis. Instead, the thumb interacts with the DNA that has been most recently synthesized.

This serves two purposes. First, it maintains the correct position of the primer and the active site. Second, the thumb helps to maintain a strong association between the DNA polymerase and its substrate. This association contributes to the ability of the DNA polymerase to add many dNTPs each time it binds a primer: template junction

Attachment of the base-paired nucleotide to the primer leads to there opening of the fingers and the movement of the primer: template junction by one base pair

DNA Polymerases Are Processive Enzymes

Catalysis by DNA polymerase is rapid. DNA polymerases are capable of adding as many as 1000 nucleotides/sec to a primer strand. The speed of DNA synthesis is largely due to the processive nature of DNA polymerase.

The role for these exonucleases became clear when it was determined that they have a strong preference to degrade DNA containing mismatched base pairs. In the rare event that an in correct nucleotide is added to the primer strand, the exonuclease removes this nucleotide from the 30 end of the primer strand. This “proofreading” of the newly added DNA gives the DNA polymerase second chance to add the correct nucleotide.

The removal of mismatched nucleotides is facilitated by the reduced ability of DNA polymerase to add a nucleotide adjacent to an incorrectly base paired primer. Mispaired DNA alters the geometry between the 30-OH and the in coming nucleotide because of poor interactions with the palm region.

This altered geometry reduces the rate of nucleotide addition in much the same way that addition of an incorrectly paired dNTP reduces catalysis. Thus, when a mismatched nucleotide is added, it both decreases the rate of new nucleotide addition and increases the rate of proofreading exonuclease activity.

In essence,proof reading exonucleases work like a “delete key,” removing only the most recent errors. The addition of a proofreading exonuclease greatly increases the accuracy of DNA synthesis.

On average, DNA polymerase inserts one incorrect nucleotide for every 105 nucleotides added. Proofreading exonucleases decrease the appearance of incorrect base pairs to1inevery107 nucleotides added. This error rate is still significantly short of the actual rate of mutation observed in a typical cell

the rare event that an incorrect nucleotide is added to the primer strand, the exonuclease removes this nucleotide from the 30 end of the primer strand. This “proofreading”of the newly added DNA gives the DNA polymerase a second chance to add the correct nucleotide. As for processive DNA synthesis,proofreading occurs without releasing the DNA from the polymerase

THE REPLICATION FORK

The junction between the newly separated template strands and the unreplicated duplex DNA is known as the replication fork. .The replication fork moves continuously toward the duplex region of unreplicated DNA, leaving in its wake two ssDNA templates that each direct the synthesis of a complementary DNA strand. The antiparallel nature of DNA creates a

ROLLING CIRCLE REPLICATION

Rolling circle replication describes a process of unidirectional nucleic acid replication that can rapidly synthesize multiple copies of circular molecules of DNA or RNA, such as plasmids, the genomes of bacteriophages, and the circular RNA genome of viroids. Some eukaryotic viruses also replicate their DNA via the rolling circle mechanism.

The model uses an asymmetric mode of replication and thus can employ the *E. coli* DNA polymerase or analogous enzymes. The synthesis begins by opening one strand of the original circle at a specific point. We imagine that the positive strand is opened, that the newly exposed 5′ end is attached to the 'membrane,' and that a new copy of this strand is synthesized by chain elongation of the 3′ end of the old strand.

Rolling circle has basically five steps:

1. DNA will be "nicked".
2. The 3' end is elongated using "unnicked" DNA as leading strand (template); 5' end is displaced.
3. Displaced DNA is a lagging strand and is made double stranded via a series of Okazaki fragments.
4. Replication of both "unnicked" and displaced DNA completes.
5. Displaced DNA circularizes.

INITIATION

Rolling circle DNA replication is initiated by an initiator protein encoded by the plasmid or bacteriophage DNA, which nicks one strand of the double-stranded, circular DNA molecule at a site called the double-strand origin, or DSO. The initiator protein remains bound to the 5' phosphate end of the nicked strand, and the free 3' hydroxyl end is released to serve as a primer for DNA synthesis by DNA polymerase III. Using the unnicked strand as a template,

- DNA synthesis takes place in 5’ to 3’ direction towards the **replication fork**. The strand that is being synthesized in this direction continuously is called the **leading strand** while the strand that is synthesized in the opposite direction is called **lagging strand**
- The leading strand requires just 1 primer whereas the lagging strand requires many such primers. Since, leading strand is synthesized continuously and simultaneously along with the discontinuous synthesis of lagging strand, the entire process of DNA synthesis is a **semi-discontinuous process**
- Fragments of lagging strand are known as **okazaki fragments**. After the strands have been completely synthesized, these okazaki fragments are joined together.
- DNA pol III is removed. DNA pol I removes the primers by its 5’ to 3’ exonuclease activity exposing the template nucleotides. It then adds complementary nucleotides by its 5’ to 3’ polymerase activity to the 3’-OH end of the previous okazaki fragment, thereby replacing the primers
- The nicks that remain behind are joined by **ligase** by creating a phosphodiester bond. This is known as nick translation
- Any errors in base-pairing is removed by DNA polymerase III by its 3’ to 5’ exonuclease activity immediately before proceeding onto the next nucleotide. This is called **proof-reading**
- Termination of this process occurs when the replication forks reach the **ter sites**. Tus proteins (Terminus Utilization Substance) bind to ter sites and halt progression of forks. In *coli*, there are 10 replication termini (Ter sites) each spanning 23 bp
- Ter B and C terminate the clockwise fork while ter A, D and E terminate anti-clockwise fork
- In circular chromosomes, the daughter chromosomes remain interlocked and are called **catenanes**. Topoisomerase II resolves this problem by breaking some bonds in DNA molecules so as to separate the strands – **Decatenation**