The role of polymerases in copying nucleic acids

Thread-Spinning Hands

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Dr. Anna Bębenek runs the Laboratory of Molecular Biology at the PAS Institute of Biochemistry and Biophysics. The laboratory studies the mechanisms and fidelity of DNA replication. She investigates the interactions between replisome proteins, and how the ends of starter molecules are transferred between the polymerase and exonuclease domains.

Accurate replication of nucleic acids (RNA and DNA) is a key process for cells. This intricate, precise job is handled by polymerases, whose specific structure allows them to build new nucleic acid strands using a single-strand matrix

The earliest reports on the existence of enzymes able to synthesize DNA were published in 1958, when Arthur Kornberg's group described a polymerase isolated from *Escherichia coli*, naming it polymerase I (Pol I). Other research teams soon discovered that bacteria and eukaryotes have numerous different polymerases, each one with distinctive properties. The rapid development of sequencing techniques towards the end of the 20th century has meant that five distinct polymerases have been described in bacteria, six in yeast, and sixteen in mammals. They have been divided into seven families on the basis of their amino acid sequences.

Family A includes all polymerases similar to Pol I. One of them is the Taq polymerase, routinely used for gene replication in molecular biology laboratories. Family B mainly includes polymerases responsible for genome replication in eukaryotic cells (for example human) and polymerases of bacteriophages - viruses that infect bacteria. Family C mainly includes the replicative polymerase of *E. coli*. The remaining families (X, Y, RT) include polymerases with very specific functions, such as enzymes found in certain viruses able to synthesize DNA strands on an RNA matrix, or telomerases adding nucleotides to the 3' end of telomeres.

The diversity of polymerases goes hand-in-hand with the broad spectrum of their function; for example, polymerases from families X and Y, and some from family A, participate in DNA repair processes. Their role is to fill gaps formed by repair enzymes, or to synthesize replacements for damaged DNA fragments. However, the most important function of polymerases is replicating genetic material, which is then transferred to new cells. The process involves polymerases from families A and B. As well as the polymerase domain - the basic structural unit - they also contain a domain which corrects wrong nucleotides inserted from the 3' end of the newly synthesized chain.

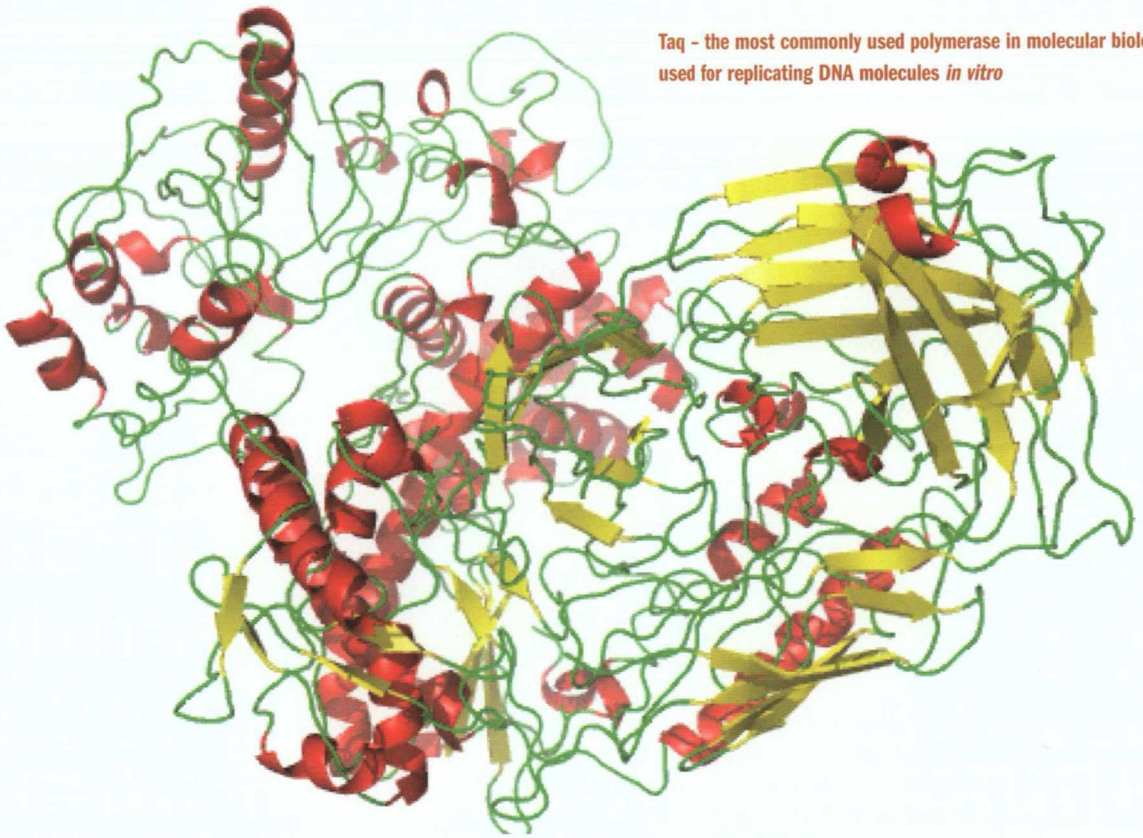
Almost error-free

Before replication can begin, the DNA helix needs to be unraveled at the site where replication is to be initiated. The DNA strand is always read in a single direction, starting from 3' towards 5'. When the helical structure is unraveled, the enzyme primase synthesizes short RNA fragments, which act as starters for the polymerase. In this respect, polymerase is a bit like a crude attempt at knitting - it can only proceed if someone starts the process. The polymerase recognizes the end of the RNA strand started by the primase, and uses the location to begin adding subsequent nucleotides - the DNA subunits complementary to the nucleotides forming the sequence in the matrix, or the DNA strand acting as the template. DNA synthesis always proceeds in the direction 5' towards 3'. The molecules comprising the new DNA strand, acting as the substrate for the polymerase, are deoxynucleotide phosphates (dNTP), inserted into the growing chain. In chemical terms, the reaction involves the formation of a phosphodiester bond between the alpha phosphate of the new nucleotide and the 3'-OH group of the final nucleotide of the starter. This is accompanied by hydrolysis of the phosphate bond and the freeing of two phosphate groups known as pyrophosphate.

But polymerase activity is not error-free. At the stage of adding nucleotides, it makes one mistake per 10^5-10^6 replicated bases. Corrective activity reduces the error rate between a hundred- and thousand-fold, depending on the type of polymerase; as a result, an incorrect nucleotide is inserted in the new DNA thread once per 10^8-10^9 bases.

Polymerase fingers

Polymerase structure was first elucidated by Tom Steitz in 1985; he obtained the first crystallographic structure of the Klenow fragment of Pol I in *E. coli*. The configurations of representatives of almost all polymerase families have now been determined. It turns



Taq - the most commonly used polymerase in molecular biology labs, used for replicating DNA molecules *in vitro*

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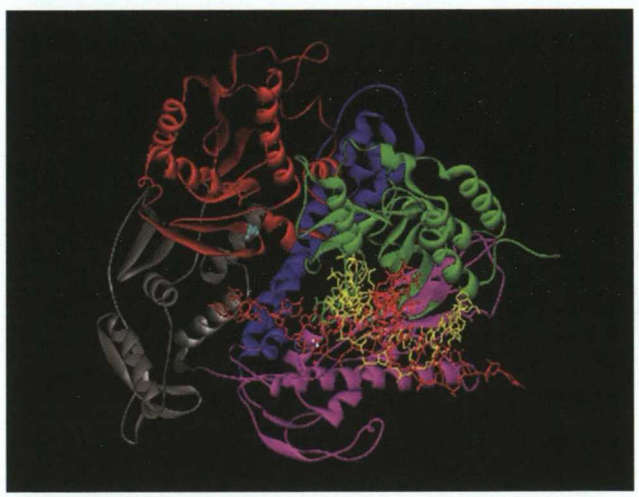
out that regardless of the differences in amino acid sequence, all polymerases share a similar basic structure: they resemble a half-open hand, with thumb, finger and palm domains.

All polymerases described thus far have active centers in the palm domain. They are responsible for binding the end of the starter and the phosphate of the nucleotide being added. The location is highly conserved, which means that its structure has evolved extremely slowly. In spite of certain differences on the level of amino acid sequence, the structures of palm domains are similar in different polymerases, which means that all polymerases use the same mechanism of nucleotide transfer.

Although the structures of thumb and finger domains vary among the polymerase families, the domains play a similar function. The finger domain plays a part in interactions between the polymerase and the single-strand matrix DNA and the new nucleotide, or building block of the new DNA strand. The finger domain is conserved in each polymerase family. In turn, the thumb domain interacts with double-stranded DNA and is likely to play an important role in the correct placement of matrix DNA in the active center of the polymerase. It also affects polymerase processivity, and plays a role in its movement along the DNA matrix.

Make no mistake!

Our understanding of how polymerase works is based on kinetic research and comparative studies of the crystal



RB69 polymerase structure – a triple complex of the polymerase and DNA matrix starter with the new nucleotide. The finger domain is marked in blue, the palm domain in purple, the thumb domain in green, the exonuclease domain in red, and the N-end domain in grey. The structure also shows the DNA of the matrix (red) and the starter (yellow) bound in the active center of the polymerase. The image does not show the new nucleotide.

structures of complexes comprising polymerase, DNA and dNTP, against their respective complexes of polymerase bound with DNA only. Such work has led to the discovery of how polymerase distinguishes between the right and wrong nucleotides. It turns out that binding dNTP results in significant changes to the spatial orientation of DNA and the finger domain, creating a space for the new

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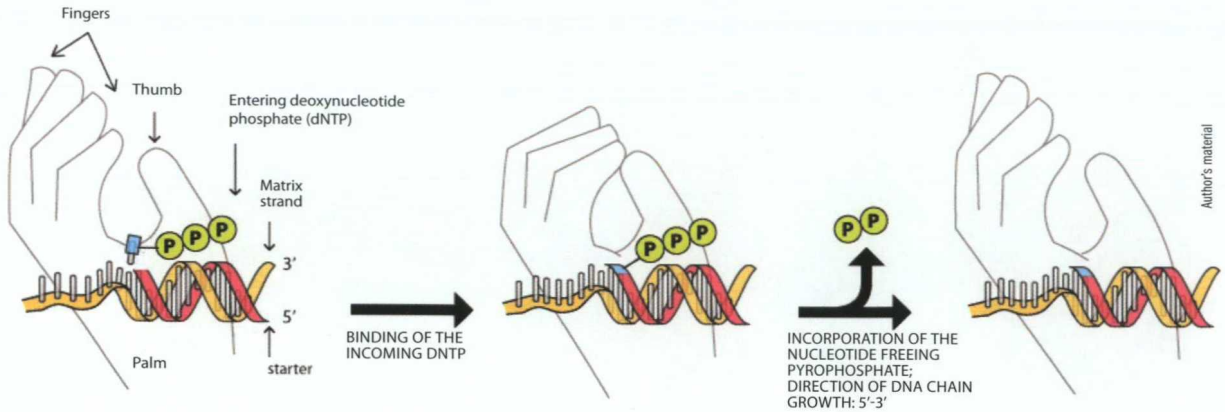
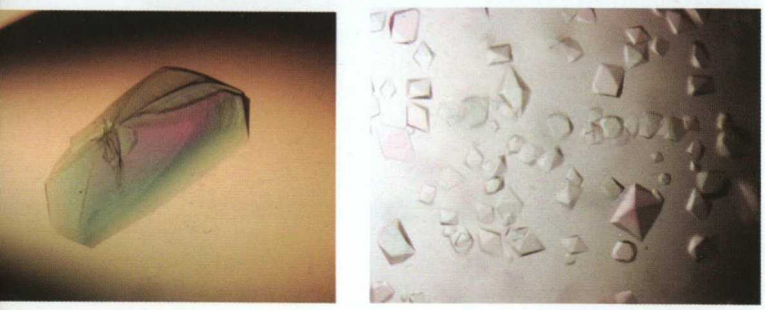


The polymerization reaction involves binding dNTP complementary to the matrix with the end of the starter. The polymerase is depicted as a half-open hand with finger, thumb and palm domains. Adding a new nucleotide is accompanied by a change of conformation from open to closed while the nucleotide is bound in the active center; the polymerase opens again after the nucleotide is incorporated

nucleotide and the matrix nucleotide. The palm domain closes, with the finger domain twisting towards the palm domain creating a closed conformation. This allows the finger domain to be in contact with the dNTP. They close around it and its complementary matrix base, creating a pocket. Its geometry means that only the right nucleotide, corresponding to the matrix nucleotide, can be bound. If the starter is bound to the wrong nucleotide, then either the finger domain opens and searches for a new, correct nucleotide, or - in corrective polymerases - the end of the starter reaches the active center of the exonuclease, where the incorrect nucleotide is eliminated.

Polymerases from family Y are an interesting exception. They show a low fidelity of replication in non-damaged DNA, yet they can replicate DNA with a disrupted



RB69 polymerase crystals. Selected crystals are analyzed using X-rays. X-ray diffraction imaging enables us to determine the spatial structure of the polymerase

symmetry, which cannot be replicated by replicative polymerases. The active center of these polymerases differs from other families; their pockets are significantly larger and able to recognize DNA with a changed, disrupted structure, bind it, and insert incorrect bases. This enables family Y polymerases to repair damaged DNA. The cost of low fidelity of DNA replication is the preserved continuity of DNA replication in cells.

What we see

Our understanding of the mechanisms of action and biological role of polymerases has advanced rapidly since the elucidation of their crystallographic structures. Our laboratory conducts research into RB69 polymerase, which is a model for family B polymerases. We use site-directed mutagenesis to change the amino acids which appear to be important in the interactions between the polymerase and DNA or new dNTP, and we characterize these mutant molecules in terms of their properties. We also study the fidelity of the replication they facilitate by using *in vivo* and *in vitro* genetic testing. Our results, in conjunction with those obtained by other laboratories, have helped make it possible to define the role of amino acids in the finger domain forming a part of the pocket, and the role of amino acids interacting with the phosphate of the nucleotide being added by the polymerase. We have also determined the regions of the polymerase responsible for interacting with DNA and binding the polymerase to the substrate. Finally, we conduct studies on polymerases from other families; the results we have obtained have greatly improved our understanding of the function of these polymerases in cells. ■

Further reading:

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