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Accuracy, lesion bypass, strand displacement and translocation by DNA polymerases

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The structures of DNA polymerases from different families show common features and significant differences that shed light on the ability of these enzymes to accurately copy DNA and translocate. The structure of a B family DNA polymerase from phage RB69 exhibits an active-site closing conformational change in the fingers domain upon forming a ternary complex with primer template in deoxynucleoside triphosphate. The rotation of the fingers domain α-helices by 60° upon dNTP binding is analogous to the changes seen in other families of polymerases. When the 3′ terminus is bound to the editing 3′ exonuclease active site, the orientation of the DNA helix axis changes by 40° and the thumb domain re-orients with the DNA. Structures of substrate and product complexes of T7 RNA polymerase, a structural homologue of T7 DNA polymerase, show that family polymerases use the rotation conformational change of the fingers domain to translocate down the DNA. The fingers opening rotation that results in translocation is powered by the release of the product pyrophosphate and also enables the Pol I family polymerases to function as a helicase in displacing the downstream non-template strand from the template strand.

Keywords: DNA polymerase; RNA polymerase; replication; translocation; protein structure

1. INTRODUCTION

Most DNA polymerases exhibit high fidelity in copying DNA and are able to move processively along the DNA duplex accompanied by a processivity factor (Kornberg & Baker 1992; Bedford *et al.* 1997; Steitz 1999). In the case of the DNA polymerase I family, the enzyme can also displace the template strand acting as a helicase as it translocates down the template strand. The lesion bypass polymerases, alternatively, are able to copy past DNA damage in the template strand that stops other DNA polymerases (Johnson *et al.* 1999; Friedberg & Gerlach 1999; Friedberg *et al.* 2000). Structural and biochemical studies of DNA polymerases now provide insights as to how these molecular scribes are able to achieve their task of accurately copying DNA templates into daughter strands.

The crystal structures of DNA polymerases from numerous families exhibit considerable diversity and some common features (figure 1). The overall structure of the polymerase domain has been divided into subdomains called 'fingers', 'palm' and 'thumb' (Kohlstaedt *et al.* 1992). The thumb domain interacts with the duplex product of DNA synthesis, while the fingers domain contacts the downstream DNA template strand and incoming deoxynucleoside triphosphate. The palm domain provides the residues involved in catalysis and aspects of fidelity checking. All polynucleotide polymerases catalyse the nucleotide addition using two divalent magnesium ions that are positioned by two or three carboxylate groups emanating from their palm domain (Steitz & Steitz 1993; Steitz *et al.* 1994). So far the palm catalytic domains of all DNA polymerases show one of two structures. The A family, B family and lesion bypass polymerases, as well as reverse transcriptase, all exhibit a palm domain structure that was first seen in the Klenow fragment of DNA polymerase I (Ollis *et al.* 1985*a*; Kohlstaedt *et al.* 1992; Wang *et al.* 1997; Doublié *et al.* 1998; Li *et al.* 1998*b*; Zhou *et al.* 2001). The palm domain of DNA polymerase β is the founding member of a second family of polymerases called the nucleotidyl transferase polymerases and is unrelated to that of the other polymerases (Pelletier *et al.* 1994; Steitz *et al.* 1994). What also differs among all of these DNA polymerase families are the structures of the thumb and fingers domains. In each of these families the structures of these subdomains are unique and indeed, define the subfamilies.

Crystal structures and biochemical as well as genetic studies have clearly shown that the fidelity of DNA copying by DNA polymerases is achieved through a variety of mechanisms that operate at the polymerase active site as well as through editing of incorrectly incorporated nucleotides at a distant 3′ exonuclease active site (Freemont *et al.* 1988; Beese & Steitz 1991; Beese *et al.* 1993*a*; Shamoo *et al.* 1995; Doublié *et al.* 1998; Li *et al.* 1998*b*; Franklin *et al.* 2001; Johnson *et al.* 2003). The crystal structures of DNA polymerase β with and without bound primer template and incoming deoxynucleoside triphosphate (Sawaya *et al.* 1994; Pelletier *et al.* 1994) as well as the corresponding crystal structures of T7 DNA polymerase ternary complex (Doublié *et al.* 1998) and the KlnTaq polymerase ternary complex (Li *et al.* 1998*b*) were the first to

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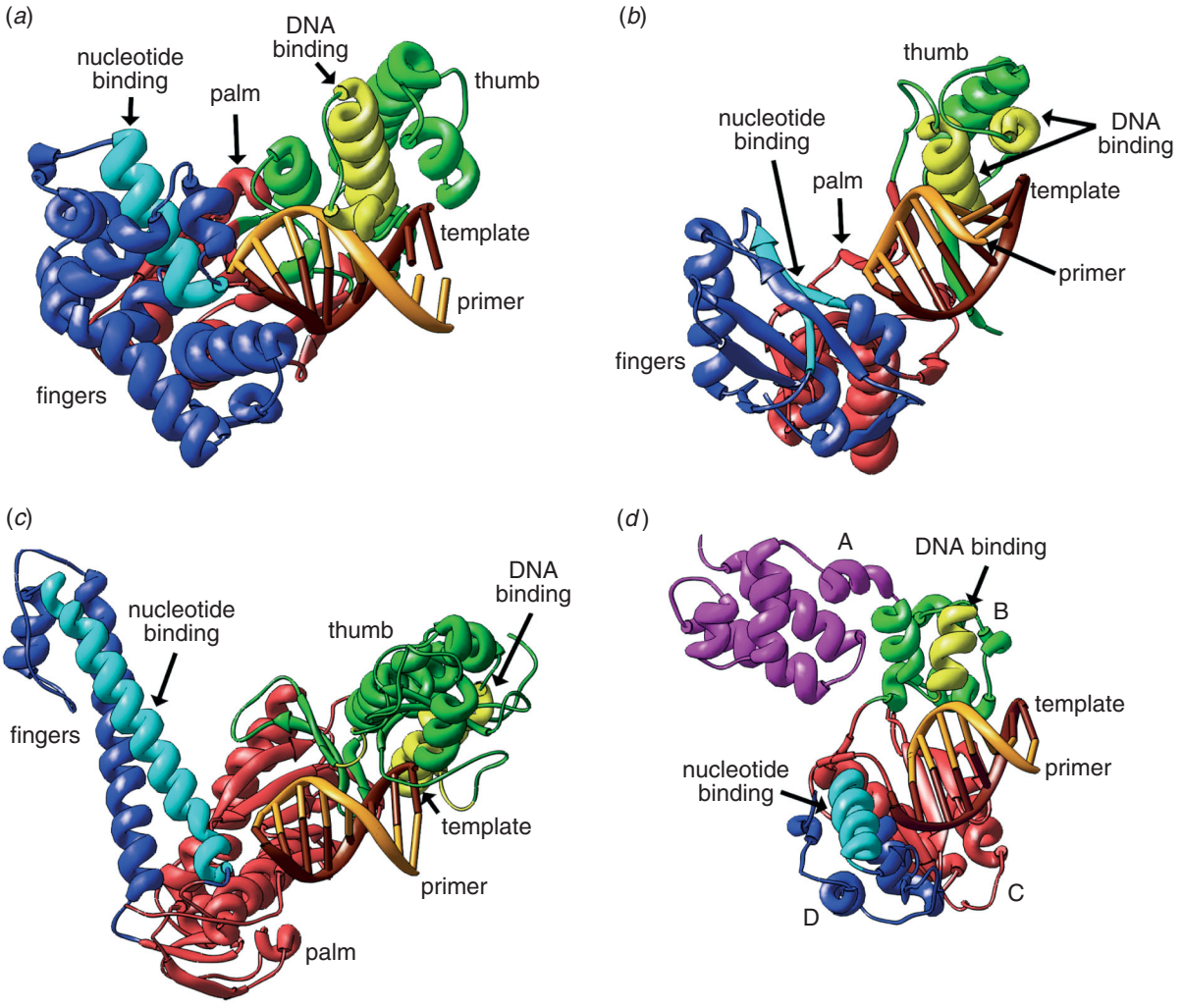

Figure 1. Comparison of the structures of polymerases from four different families (Brautigam & Steitz 1998). (*a*) Pol I or A family; (*b*) reverse transcriptase family; (*c*) B or Pol α family; and (*d*) Pol β or nucleotidyl transferase family.

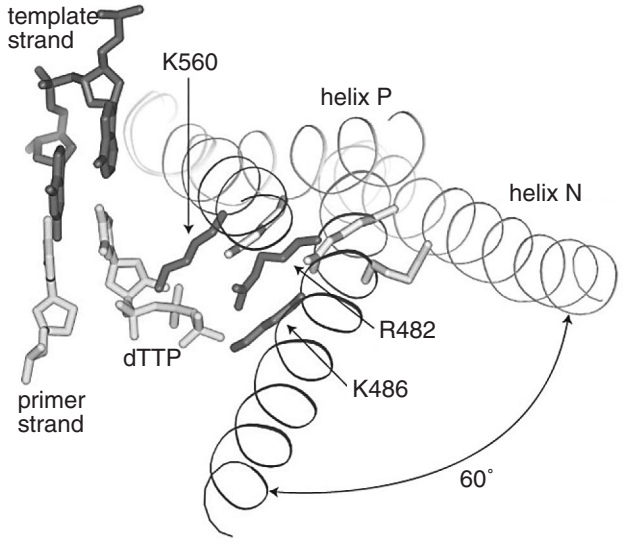

Figure 2. The DNA- and dNTP-induced conformation change in the fingers domain of RB69 DNA polymerase (Franklin *et al.* 2001).

demonstrate that formation of the correct ternary complex produced a significant conformational change in the fingers domain of these two non-homologous enzymes. In these cases it was hypothesized that this conformational change, which is essential for the catalytic phosphoryl transfer reaction, will occur only when the incoming nucleotide forms a Watson–Crick base pair with the template strand. Any non-Watson–Crick base pair that is formed does not fit in the 'closed' conformation required for a catalytically active ternary complex. It also appears that the formation of a non-Watson–Crick base pair misorients the 3′ terminus of the primer strand thereby stalling further nucleotide incorporation by the polymerase.

The misincorporation of a nucleotide not only leads to a stalling of the polymerase-catalysed addition of the next nucleotide, but it also destabilizes the duplex thereby facilitating the formation of a partly single-stranded 3′ terminus that is capable of moving to the editing exonuclease active site. In the case of DNA polymerase I, four nucleotides at the 3′ terminus peel off the template strand and are observed to bind in the exonuclease active site located on an adjacent domain *ca.* 35 angstroms (Å, where 1 Å $= 1 \times 10^{-10}$ m) from the polymerase active site (Beese *et al.* 1993*a*). Although excision of the 3′ nucleotide occurs for both correctly and incorrectly incorporated

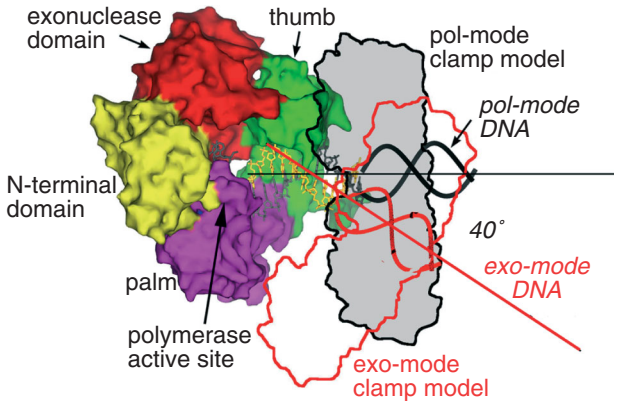

Figure 3. Superposition of primer-template DNA bound in editing mode to the exonuclease active site (red) and on that bound to the polymerase active site (black) of RB69 DNA polymerase along with the sliding clamp (Shamoo & Steitz 1999).

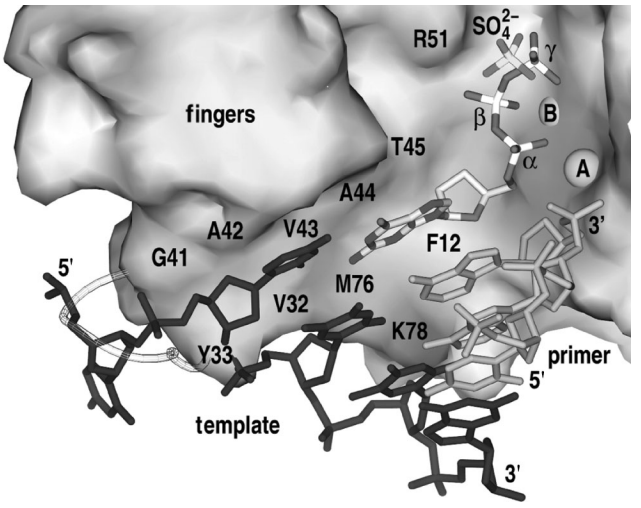

Figure 4. The structure of a DinB polymerase fragment lacking the C-terminal domain with homology modelled DNA (Zhou *et al.* 2001).

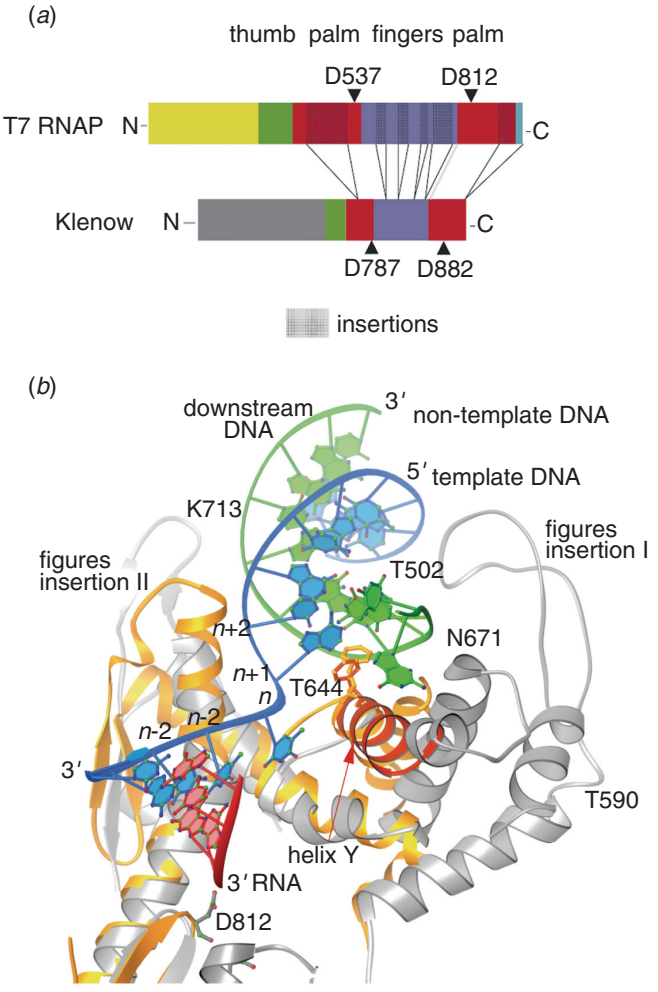

Figure 5. Structural based alignment of T7 RNAP polymerase and Klenow fragment shows similar domains arrangement in both polymerases. (*a*) Graphic representation of structural guided primary sequence alignment. (*b*) Superposition of the fingers domain of T7 RNA polymerase (grey) with bound downstream DNA on the corresponding fingers domain of the Klenow fragment (yellow; Yin & Steitz 2002).

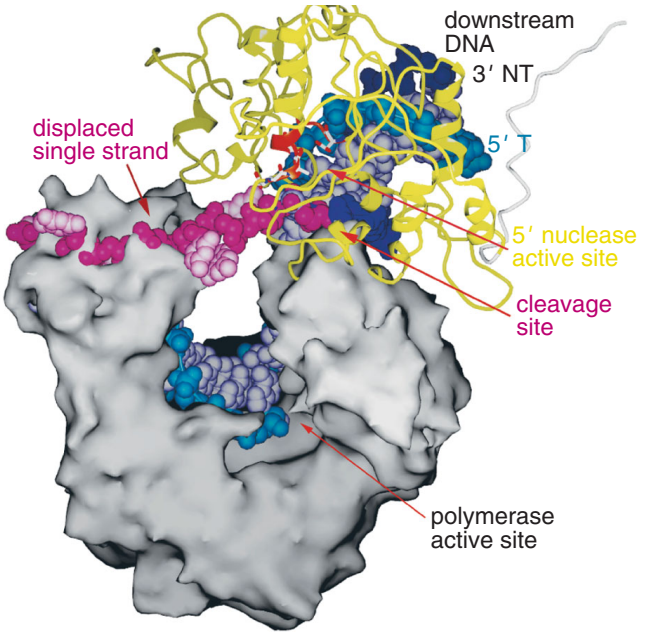

Figure 6. A model of Taq DNA polymerase with a repositioned 5′ nuclease domain with its active-site region (red) near the point of cleavage between the displaced strand (purple) and the downstream non-template strand (blue).

nucleotides, it occurs at a far higher rate for incorrectly added nucleotides due both to the stalling at the polymerase active site and the destabilization of the duplex.

2. PHAGE RB69 DNA POLYMERASE SUBSTRATE COMPLEXES

The replicative DNA polymerase from phage RB69 is a B family DNA polymerase that shows sequence homology to the human DNA polymerase α and is a close homologue of phage T4 DNA polymerase (Yeh *et al.* 1998). The crystal structures of the apo-DNA polymerase (Wang *et al.* 1997), its complex with primer-template DNA bound at the exonuclease active site (Shamoo & Steitz 1999) and that of a ternary complex of primer

template and deoxynucleoside triphosphate bound to the polymerase active site (Franklin *et al.* 2001) have been determined. As occurs in the case of DNA polymerase β, Pol I DNA polymerase and reverse transcriptase (Pelletier *et al.* 1996; Huang *et al.* 1998; Doublié *et al.* 1999), the formation of the ternary complex results in a large conformational change in the fingers domain. In the case of RB69 DNA polymerase the fingers domain consists of two anti-parallel α-helices, which rotate by *ca.* 60° in response to the binding of substrate primer-template DNA and dNTP (figure 2). This rotation appears to be facilitated by the interaction of lysine 560, arginine 484 and lysine 486, which are located on the fingers α-helices, with the three phosphates of the bound deoxynucleoside triphosphate. As in the case of other polymerases, the base pair between the nascent dNTP and the template base lies in a pocket formed by the enzyme. A mismatched base pair would not fit into this pocket thus precluding this catalytically essential conformational change. As we shall see, this rotation of the fingers domain also plays an important role in translocation.

The axis of the duplex DNA bound to the RB69 DNA polymerase exonuclease active site is rotated by *ca.* 40° relative to its orientation when bound to the polymerase active site (figure 3). Once again, as in the case of the Pol I family polymerases, the polymerase and exonuclease active sites are separated by *ca.* 35 Å (Shamoo & Steitz 1999; Franklin *et al.* 2001). Furthermore, there is a significant alteration in the conformation of the thumb domain which appears to guide the re-orientation of the DNA as it traverses from the polymerase to the exonuclease active site and back.

A model for the polymerase bound to duplex DNA that includes the sliding clamp processivity protein has been built based on a crystal structure of the sliding clamp with a C-terminal peptide from the RB69 polymerase (Shamoo & Steitz 1999). The extreme C-terminus of the polymerase protrudes from the body of the enzyme in a location that suggested it might interact with the sliding clamp (Wang *et al.* 1997). Proteolysis experiments that remove the extreme C-terminus remove the interaction between the polymerase and the processivity factor, rendering the polymerase non-processive (Bernad *et al.* 1990; Goodrich *et al.* 1997). The co-crystal structure of the clamp and a short C-terminal polymerase peptide shows the peptide bound into a pocket in the clamp. The clamp protein can be docked onto the polymerase by superimposing the C-terminus of the polymerase on the peptide as bound to the clamp (Shamoo & Steitz 1999). This orientation allows the DNA that is bound to polymerase to pass through the centre in the clamp protein. The fact that the DNA changes its orientation in going from the exonuclease to the polymerase active site suggests that likewise the clamp must change it orientation relative to the polymerase as the DNA moves back and forth, a requirement that is made possible by its attachment by a flexible C-terminal tail (figure 3).

3. DNA LESION BYPASS POLYMERASES

Y-family DNA lesion bypass polymerases are found in eubacteria, eukaryotes and archaea and are hypothesized to replace replicative DNA polymerases that are stalled at sites of unrepaired DNA damage (Woodgate 1999; Friedberg & Gerlach 1999; Goodman & Tippin 2000). They have the common features of low fidelity on undamaged DNA, low processivity and no intrinsic exonuclease activity. The DinB lesion bypass polymerase, for example, is able to bypass the benzo[a]pyrene adduct of guanosine, a carcinogen found in tobacco smoke. The crystal structure of the DinB homologue from *Sulpholobus solfactaricus* was the first lesion bypass polymerase whose structure was determined and it provides insights into how these enzymes are able to bypass lesions (Zhou *et al.* 2001).

The structure of a DinB lesion bypass polymerase fragment that includes the polymerase domain but lacks the C-terminal domain was determined at 2.3 Å resolution (Zhou *et al.* 2001). Although a relationship was not recognized from sequence comparisons, the structure of the palm domain is nearly identical to that of Pol I and most other DNA polymerase families. The fingers and thumb domains, however, have largely novel structures. Homology modelling of primer-template DNA onto the DinB enzyme using the known structures of ternary complexes suggests that there is a comparatively small surface area of contact between protein and DNA and that the binding pocket for the nascent base pair is quite open and unconstrained (figure 4). It appears that the fingers domain of the apo-enzyme is already oriented in the conformation that is characteristic of a ternary polymerase complexes. The open, unconstraining structure surrounding the nascent base pair would allow for accommodation of DNA lesions in the template strand. The apparent lack of a need for a fingers conformational change induced by the formation of the nascent base pair suggests that the error recognition mechanism characteristic of other polymerase active sites is not functional in this enzyme. The subsequent crystal structure of a complete lesion bypass enzyme complexed with DNA primer template is completely consistent with these conclusions (Ling *et al.* 2001).

4. TRANSLOCATION AND STRAND DISPLACEMENT

The extensive structural homology between T7 RNA polymerase and the Pol I family of DNA polymerases allows studies of the former enzyme to enlighten processes that are also carried out by the latter enzymes. The RNA polymerase from bacteriophage T7 is a 98 000 Da molecular mass monomer whose C-terminal two-thirds shows significant sequence and structural homology to the Pol I family DNA polymerases (figure 5*a*), including the T7 DNA polymerase (Ollis *et al.* 1985*b*). The N-terminal third of the protein forms unique fold that plays a role in promoter recognition and the enzyme's conversion from initiation to elongation states of transcription (Cheetham *et al.* 1998; Cheetham & Steitz 1999; Yin & Steitz 2002). Recent crystal structures of elongation state complexes between the T7 RNA polymerase and a 30 base pair DNA duplex, a 17 nucleotide RNA transcript and various nucleotide substrates provide insights into the mechanisms by which the product duplex is translocated after nucleotide incorporation and the downstream DNA strands are separated. A structural homology between the T7 RNA and DNA polymerases as well as *Escherichia coli* DNA polymerase I allows understanding of how DNA polymerase I

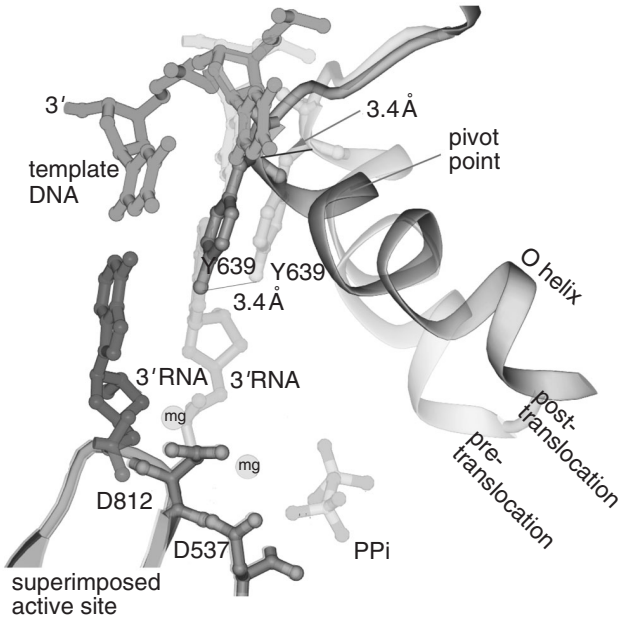

Figure 7. Superposition of the pre- and post-translocation structures shows that the fingers 'O' helix rotation results in duplex translocation. This fingers rotation is powered by pyrophosphate dissociation. The structure of the pre-translocation complex (light grey, background structure) with bound pyrophosphate crosslinking the basic residues of 'O' helix to a magnesium ion bound to the catalytic carboxylates. The structure of a post-translocation complex (darker grey, foreground structure) formed after pyrophosphate dissociation with the resulting pivoting motion of the 'O' helix indicated.

is able to accomplish DNA synthesis with displacement of the non-template strand and how the 5′ nuclease is able to cleave the non-template strand leaving only a nick between the upstream DNA and the newly synthesized DNA primer terminus.

5. DOWNSTREAM NON-TEMPLATE STRAND DISPLACEMENT

It is now possible to provide a structural basis for understanding the ability of DNA polymerase I to not only fill single-stranded gaps in DNA duplexes, but also to displace the RNA primers of Okazaki fragments while synthesizing DNA (strand displacement), leaving only a nick in the DNA duplex (nick translation). Superposition of the Cα backbones of the palm domains of T7 RNA polymerase and *E. coli* Klenow fragment aligns the homologous portions of the respective fingers domains, and the downstream duplex DNA bound to T7 RNAP fits well onto the fingers domain of Klenow fragment (figure 5*b*). Corresponding homologous α-helices in the two polymerases align precisely and lie between the template and non-template strands at the point of their separation, and a conserved phenylalanine stacks on the last template strand base of the downstream duplex. Translocation of the DNA product after nucleotide incorporation results in the strands of the downstream DNA being separated by the α-helix lying between them.

In the model of DNA polymerase I containing bound downstream DNA that was constructed by homology modelling, the non-template strand departs the Pol I downstream duplex in the direction of the 5′ nuclease domain which is responsible for cleaving the Okazaki RNA. The template strand enters the RNA polymerase active site in the same way as the template strand of DNA in the Pol I DNA polymerase ternary complexes (Doublié *et al.* 1998; Kiefer *et al.* 1998; Yin & Steitz 2002). Owing to a nearly 90° kink in the direction of the downstream DNA and the direction of the newly synthesized DNA duplex, the position of the non-template strand to be cleaved by the 5′ nuclease is directed away from the polymerase and lies in a position that could be contacted by the nuclease. Remodelling the structure of Taq polymerase allows repositioning of the 5′ nuclease active site close to the expected cleavage site (figure 6). While the protein linker that connects the 5′ nuclease domain to the 3′ nuclease domain (and is protease sensitive) must be repositioned from its location as observed in the apo Taq polymerase structure, it is long enough to span the required distance. Furthermore, the newly formed base pair after nucleotide incorporation and the last base pair in the downstream DNA are adjacent to each other in the template sequence. Thus, after cleavage of the displaced non-template strand by the 5′ nuclease, only a nick remains between the 5′ phosphate of the cleaved strand and the 3′ primer strand nucleotide.

6. PPi RELEASE AND TRANSLOCATION

All polymerases must translocate down the template strand subsequent to the incorporation of the nucleoside or deoxynucleoside triphosphate. One could imagine that this translocation might happen in a distributive mode of product dissociation (either in one dimension or in three dimensions) and rebinding at the substrate site. Alternatively, the energy available from deoxynucleoside triphosphate incorporation could be coupled to an active translocation of the primer-template DNA in a ratchet-like mechanism. The crystal structures of various T7 RNA polymerase elongation complexes are consistent with the latter hypothesis.

The crystal structures of three elongation complexes with T7 RNA polymerase shed light on the translocation process and suggest that it is the dissociation of the pyrophosphate product of nucleotide incorporation that powers translocation. These structures represent all steps in nucleotide incorporation:

(i) a T7 RNAP ternary complex containing an incoming nucleoside triphosphate analogue, duplex DNA and an RNA transcript;
(ii) a pre-translocation binary product complex containing a pyrophosphate; and
(iii) a post-translocation product binary complex after pyrophosphate has been released.

The structures of the ternary substrate complex and the product complex in the presence of pyrophosphate are essentially identical with the product not having been translocated. The structure of the product RNA–DNA

heteroduplex after pyrophosphate release, however, shows that the product has been translocated and the structure of the enzyme has been changed by rotation of a fingers domain about a pivot point that is analogous to that seen between the open apo-enzyme and closed ternary complex of T7 DNA polymerase (Doublié *et al.* 1999). This rotation of the α-helix that is homologous to the 'O' helix of Klenow fragment not only returns to the open structure, but one end of the helix pivots 3.4 Å in the direction of the heteroduplex product and appears to affect its translocation.

It appears that the binding of NTP to the open complex initially involves interaction of the O-helix in the fingers domain with the tripolyphosphate moiety, because the template nucleotide that is to form the nascent base pair is sequestered in a pocket and a tyrosine side-chain overlaps the position that the base of the nascent NTP takes when it pairs with the template. The triphosphate and associated magnesium ions may power the conformational change in the fingers domain by allowing the formation of a bridge between the active-site carboxylates, the magnesium ions bound to the phosphates and the basic residues of the fingers, which interact with the phosphates. This conformational change repositions the nascent template base to lie opposite the incoming base and simultaneously pivots the helix and tyrosine making available the binding site of the nascent incoming nucleotide triphosphate base. The product complex in the presence of pyrophosphate shows that the closed conformation of the enzyme is maintained by the pyrophosphate product still forming the bridge between the fingers domain and the magnesium ion bound to the active-site carboxyl groups. Release of the pyrophosphate, however, eliminates this bridge leading to a reversal of the fingers conformational change to form the open complex structure. Associated with this change is a rotation of the O-helix of the fingers domain about a pivot point one-third from its end, which pushes the product duplex by 3.4 Å and results in translocation (figure 7).

These three structures of T7 RNA polymerase elongation complexes along with the structures of an open complex of DNA polymerase complexed with deoxynucleoside triphosphate (Beese *et al.* 1993*b*; Li *et al.* 1998*a*; Yin & Steitz 2002, 2004) show that both the finger closing conformational change that is induced by the correct nucleoside triphosphate but not the incorrect nucleoside triphosphate and the finger opening conformational change that results in translocation of the duplex product subsequent to nucleotide incorporation are powered by phosphates of NTP. Binding of the triphosphate moiety of NTP results in finger closure, while dissociation of the product pyrophosphate results in finger opening. The analogous conformational changes will occur with both the DNA polymerase I family and DNA polymerase α family enzymes. Furthermore, this NTP-driven conformational change that powers the translocation will also power the separation of the strands of the downstream DNA as the downstream DNA is pulled progressively upstream.

7. PERSPECTIVES

Whereas the model of duplex DNA proposed by Watson and Crick 50 years ago immediately suggested to them how DNA could be copied, the biochemical and structural mechanisms by which enzymes are able to achieve the accurate copying of DNA into daughter strands has required 50 years to work out.

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GLOSSARY

dNTP: deoxyribonucleotide triphosphate
PPi: pyrophosphate