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MINIREVIEW

What Is the Orientation of DNA Polymerases on Their Templates?

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Considerable controversy now surrounds the interpretation of the known structures of the nucleic acid polymerases and the orientation of the template-primers relative to the polymerase active sites. The initial polymerase structure (that of the Klenow fragment of *Escherichia coli* DNA polymerase I) showed a large groove into which DNA might bind but did not provide sufficient information to make clear the orientation of the DNA relative to the protein (20). This question was apparently answered by solving the structure of the reverse transcriptase (RT) of human immunodeficiency virus type 1 (HIV-1) (16). As will be discussed in more detail below, HIV-1 RT has, in addition to the polymerase domain, an RNase H domain, which can be used to define the orientation of the enzyme on an RNA/DNA duplex. The issue of the orientation of the protein relative to the nucleic acid appeared to be completely settled when the structure of HIV-1 RT in complex with double-stranded DNA was obtained (14). The orientation of the DNA in this structure is in agreement with the prediction made on the basis of the physical relationship of the polymerase and the RNase H domains. However, Pelletier et al. (22) obtained a structure for a complex of rat DNA polymerase β (pol β) and double-stranded DNA that appears to be in conflict with data obtained for HIV-1 RT—the orientation of the DNA in the pol β structure is opposite to that predicted and observed in the HIV-1 RT structure. On the basis of this observation, Pelletier et al. concluded that the structure of HIV-1 RT in complex with double-stranded DNA was an artifact, and to account for the biochemical data that defines the orientation of HIV-1 RT on an RNA template (discussed below), they proposed that HIV-1 RT is a bidirectional polymerase having opposite orientations on RNA and DNA templates. That proposal is controversial. Steitz et al. (28) have attempted to reconcile the apparent discrepancy by proposing that all of the data are correct but that the criteria used to define the orientation of the pol β protein are wrong: it really has the same orientation on DNA as HIV-1 RT, but Pelletier et al. were incorrect in the criteria they chose to define the structural and functional relationships of pol β and HIV-1 RT. We do not think either of these proposals entirely persuasive but believe it is possible to make relatively simple proposals that can reconcile most of the data. In making these proposals, we were influenced by the need to preserve a unified mechanism for polymerization and by the considerable similarities in the structure of the regions that include the active sites of all the polymerases whose three-dimensional structures are known. However, it does appear, on the basis both of sequence comparisons and a recently reported structural analysis, that pol β is evolutionarily distinct from the other polymerases whose structures have been solved.

We will begin by reviewing the data that define the orientation of HIV-1 RT on RNA and DNA templates and then, in this light, consider the proposals of Pelletier et al. (22) and Steitz et al. (28). Four sets of data relevant to the orientation of RNA and DNA templates on HIV-1 RT will be considered: (i) data suggesting that the direction of HIV-1 RT polymerization is the same for both RNA and DNA templates, (ii) data that give the absolute orientation of RT on an RNA template, (iii) data providing the absolute orientation of RT on a DNA template, and (iv) modeling experiments in which attempts are made to bind DNA to RT in the pol β orientation.

The simplest argument that RT is oriented in the same direction on RNA and DNA templates is that the vast majority of point mutations in HIV-1 RT have quite similar effects on the polymerase activity with both templates. Of several hundred point mutations of HIV-1 RT that have been tested, only a handful behave differently on RNA and DNA templates (3-5).

As part of their proposal, Pelletier et al. (22) specifically suggested that when HIV-1 RT copies a DNA template, Tyr-183 in the highly conserved YMDD motif plays the same role that the catalytically essential Asp-110 does when RNA templates are copied (Fig. 1). However, an HIV-1 RT variant carrying an Asp-110→Glu mutation was unable to copy either RNA or DNA templates (the mutant enzyme retains less than 5% of the activity of the wild-type enzyme), yet a Tyr-183→Phe HIV-1 RT mutant can copy either template with similar efficiencies (approximately one-third that of the wild-type enzyme) (3). Substituting Phe for Tyr at position 183 removes the hydroxyl which the hypothesis suggests would be essential for DNA-dependent DNA polymerase activity in the model proposed by Pelletier et al. (22). Similar arguments can be made for a number of other mutations (3-5). Although these data do not, by themselves, give information about the absolute orientation of HIV-1 RT, they do suggest that the orientation is the same on both RNA and DNA templates.

The absolute orientation of RT on RNA templates can be deduced from experiments that measure the relative positions of the polymerase and RNase H active sites. During polymerization on an RNA template, the HIV-1 RNase H active site

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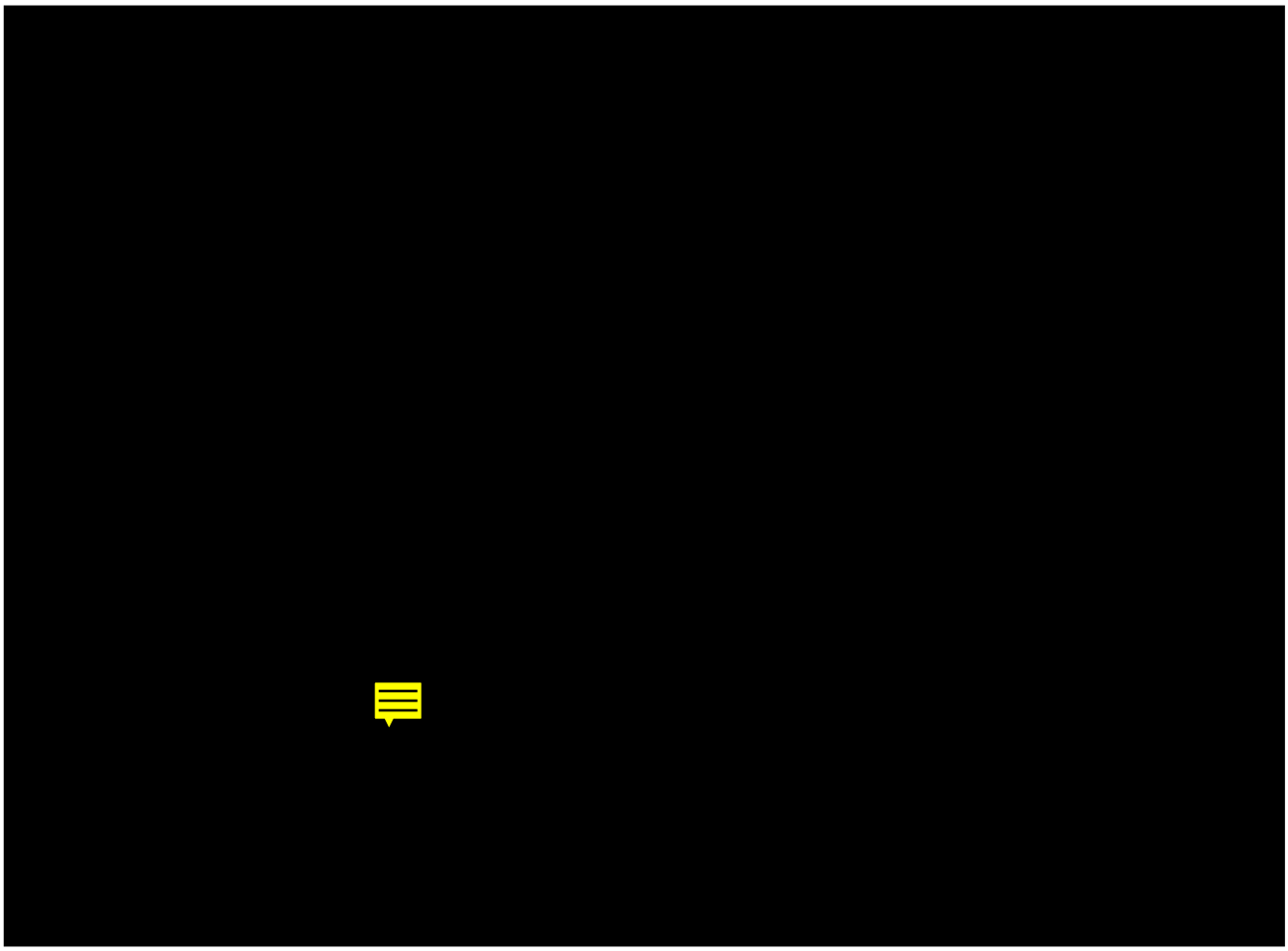

FIG. 1. Comparison of the superposition of the DNA from the pol β-DNA-ddCTP structure (22) on the HIV-1 RT-Fab-DNA structure (14) and the DNA from the HIV-1 RT Fab/DNA structure on the pol β/DNA/ddCTP structure. The pol β-DNA-ddCTP complex and the HIV-1 RT-Fab-DNA complexes were aligned by superimposing the corresponding Cα positions of two α-helices and two β-strands in the palm subdomains of these enzymes, as done by Pelletier et al. (22). (A) The DNA of the pol β complex is shown superimposed on the HIV-1 RT complex. The pol β DNA is on the left and the RT DNA is on the right. The template strands are colored magenta, and the primer strands are purple. The subdomains of the p66 subunit of HIV-1 RT are colored as follows: fingers (p66F), blue; palm (p66P), red; thumb (p66T), green; connection (p66C), yellow; RNase H (p66H), orange. The entire RT p51 subunit is colored gray. The positions of the three essential catalytic aspartic acid residues (D110, D185, and D186) are shown as cyan-colored spheres. This panel illustrates how the DNA in the HIV-1 RT-Fab-DNA complex lies in the cleft formed by the fingers, palm, and thumb subdomains of HIV-1 RT and spans the region between the polymerase and the RNase H sites. In contrast, the DNA from the pol β structure makes only relatively minor contacts with HIV-1 RT. (B) DNA of the HIV-1 RT complex is shown superimposed on the pol β complex in a mode such that DNA resembles the upstream portion of a gapped duplex which is the natural substrate for pol β. The orientation and color of the DNA strands and fingers, palm, and thumb subdomains of pol β are as described for panel A. The three essential catalytic aspartic acid residues (D190, D192, and D256) of pol β are shown as cyan-colored spheres. A minor rotation of the 8-kDa domain in the direction of the arrow would bring the 8-kDa domain involved in DNA binding into extensive contact with the double-stranded portion of the template-primer of the RT DNA. The flexibility of the hinge region connecting the catalytic and 8-kDa domains of pol β should permit this rotation. (C) Close-up of the palm of HIV-1 RT showing the side chains of the amino acids in the conserved YMDD motif and their relation to D110, which together with D185 and D186 forms the polymerase active site, and the 3′ termini of the primer strands of the DNA from the pol β structure (left) and RT DNA (right). (D) Close-up of the palm of pol β showing the spatial relationship of the three catalytic aspartic acid residues (D190, D192, and D256) and the 3′ primer termini of the DNA of the pol β and RT complexes. Also shown is the location of αM of the thumb domain.

trails the polymerase active site by some 14 to 18 bp (9, 12, 33). This is the logical orientation for HIV-1 RT, since it permits cleavage of the RNA (template) strand of the newly formed RNA/DNA duplex.

The strongest argument for the orientation of HIV-1 RT on DNA templates comes from the structure of the HIV-1 RT-DNA complex (14). In this structure the 18-bp DNA duplex region occupies the binding cleft formed by the fingers, palm, and thumb subdomains of HIV-1 RT and spans the region between the polymerase and RNase H active sites (Fig. 1). This orientation of HIV-1 RT provides the enzyme with an opportunity to grasp and hold the relatively rigid duplex region of the template-primer and, in so doing, positions the 3′-OH of the primer terminus at the polymerase active site. Further evidence that the 3′-OH is in the correct orientation relative to the polymerase active site comes from observations that a mercurated deoxynucleoside triphosphate can be bound to the polymerase active site in this complex (2, 14).

Recent footprinting experiments indicate that about 18 to 24 bp of duplex DNA are protected by HIV-1 RT (19, 33), which is in good agreement with predictions based on the separation of polymerase and RNase H active sites. If the DNA were in the orientation suggested by Pelletier et al. (22), relatively few base pairs (approximately six to eight) would be protected from DNase I cleavage by HIV-1 RT. Furthermore, HIV-1 RT mutants containing small deletions in RNase H gave slightly

smaller footprints on duplex DNA than did wild-type enzyme. These results unambiguously define the position of RNase H on the DNA duplex (11). Footprinting of the HIV-1 RT-DNA complexes with S1 nuclease suggests that a single-stranded region of about six nucleotides is shielded by HIV-1 RT, which is consistent with predicted interactions of the portion of the single-stranded template that would interact with the p66 fingers subdomain (34). Not surprisingly, the results of all of the chemical and enzymatic footprinting experiments support the structure of the HIV-1 RT-DNA complex seen in the crystals. Additional footprinting experiments have been done with equine infectious anemia virus RT (25) and murine leukemia virus (MLV) RT (32); it is clear from the experiments that these (and presumably all other) retroviral RTs bind DNA in the same orientation.

In addition, substantial problems arise if attempts are made to model the binding of DNA to HIV-1 RT in the orientation proposed by Pelletier et al. (8a, 22) (Fig. 1). Orienting DNA in this fashion causes serious steric conflicts between the template strand of the DNA duplex and the β-sheet structure in the HIV-1 RT palm subdomain (β12, β13, and β14). It is not possible to generate contacts between residues of the β-sheet containing the HIV-1 RT catalytic site and the template strand without gross movement of the β12-β13-β14 sheet, which seems unlikely since this region is in a similar position in all of the known HIV-1 RT crystal structures whether or not double-stranded DNA is present. Although comparison of the structures shows that portions of the β12-β13-β14 sheet differ by 2 to 3 Å (1 Å = 0.1 nm) in the available HIV-1 RT structures, a much larger movement would be required to accommodate the template-primer in the orientations proposed by Pelletier et al. (22). In addition, very little of the binding cleft of HIV-1 RT that was both predicted and observed to be the site of duplex binding (14, 16) would be in contact with the DNA if the pol β model is applied to HIV-1 RT. Other than a possible interaction with the tip of the β3-β4 loop of the HIV-1 RT fingers subdomain, the primer strand of the DNA makes essentially no contact with the enzyme. Moreover, some of the most highly conserved amino acids found in all RNA-dependent polymerases, those in motif B of Delarue et al. (8) and Poch et al. (23), are not proximal to the template-primer if the DNA is oriented and positioned according to the system of Pelletier et al. (22). In the crystal structure of the HIV-1 RT-DNA complex, these elements of the p66 fingers and palm subdomains form part of the template grip and serve to position the template-primer (14). Genetic and biochemical data support such a role for these residues (5, 6).

The regions of the palm domains near the polymerase active site of three of the four enzymes (the Klenow fragment of *E. coli* DNA polymerase I, T7 RNA polymerase, and HIV-1 RT) are sufficiently similar that it is generally accepted that these three structures are functionally related. After the original submission of this report, three new structures, those of *Taq* DNA polymerase (15), of a large fragment of *Taq* DNA polymerase, called Klentaq 1, which is analogous to the Klenow fragment of *E. coli* DNA polymerase I (17), and of a fragment of Moloney MLV RT (10) were reported. As would be expected from amino acid sequence comparisons, all of these structures have a similar architecture at the polymerase active site.

Is the structure near the active site of pol β functionally analogous to those of the other polymerases or is it only superficially similar? Unlike the other polymerases whose structures have been determined, pol β is not a replicative polymerase, but rather a distributive polymerase that fills in small gaps in DNA. DNA polymerases are commonly classified into four families on the basis of sequence similarity. Delarue et al. (8) first recognized, and Patel et al. (21) later noted, that pol β is most similar in sequence to a nucleotidyl-transferring enzyme, deoxynucleotidyltransferase. The significance of this sequence similarity is further supported by the analysis of Holm and Sander (13). The folding of the protein domains that contain the active sites of kanamycin nucleotidyltransferase and DNA pol β are strikingly similar, although there is only a 10% sequence identity over the 81 residues that form the conserved structure. The folding topology and structures of the kanamycin nucleotidyltransferase and the palm subdomain of pol β are sufficiently similar to suggest that they diverged from a common ancestor.

Unge et al. (30) and Georgiadis et al. (10) have suggested that the palm subdomains of polymerases other than pol β have folding topologies and three-dimensional structures that are related to the ribonucleoprotein (RNP) motif (βαββαβ) present in a number of RNA binding proteins. On the basis of their conserved architectures and related functions, it appears reasonable to suggest that these polymerases and the RNA binding motif are evolutionarily related. The folding topology of these domains, all of which contain a four-stranded, antiparallel β-sheet, appears to be distinct from that of the pol β palm subdomain and the kanamycin nucleotidyltransferase structures which have a mixed parallal and antiparallel β-sheet structure.

This suggests that pol β belongs to a distinct family of polymerases. It is possible, therefore, that pol β has a different mode of template-primer binding than the other polymerases and that the orientation of the nucleic acid relative to its palm subdomain may also be different. Whether this is true, there are substantial differences between pol β and the other polymerases, and it is inappropriate to interpret this complex as the prototype of a template-primer polymerase complex, with mechanistic interpretations for DNA polymerases in general and for HIV-1 RT in particular.

Although the polymerase active site region of pol β is not as closely related to the polymerase active site regions of the other four polymerases as they are to each other, considerable similarities do exist (for example, all polymerase active sites share a common αββα motif that is topologically equivalent). Davies et al. (7) and Sawaya et al. (26) both proposed that the overall similarity in the arrangement of the secondary structure can be used as a basis for structural alignment. This alignment leads to the superposition of the three catalytically critical carboxylate residues common to all the polymerases whose structures are known, which presumably reflects common structural requirements for DNA synthesis.

Steitz et al. (28) have an alternative interpretation of the structural data which is based on superposition of other functionally important elements in the structure: the two divalent metal ions in the catalytic site 3′ terminus and of the primer. This proposal remedies the apparent inconsistency in the observed orientation of the template-primer but at the expense of discounting similarities in protein architecture of the catalytic sites, which are lost in this alignment. Although it is difficult to disregard the apparent similarities in the structures of pol β and the other polymerases, if pol β has a distinct evolutionary origin, the apparent similarity in the structures could be a coincidence.

Pelletier et al. (22) have suggested, in an attempt to rationalize the discrepancy between the postulated direction of DNA synthesis by pol β and that derived from the structures of Klenow fragment and HIV-1 RT complexed with DNA, that the binding modes of DNA for Klenow fragment and for HIV-1 RT seen in the crystal complexes are not biologically

relevant. In fact, there are several features in the pol β ternary complex that cannot be easily reconciled with the available biochemical data. The following points should be considered when discussing the biological relevance of this structure.

(i) The fact that the 6-nucleotide primer strand was extended by 1 nucleotide under the crystallization conditions (to give the 11 by 7-nucleotide fragment actually present in the crystals) does not necessarily mean that the complex present in the crystals is the form that catalyzed the DNA synthesis. Since polymerization by pol β on single-stranded substrates is expected to be distributive (1) and the components of the reaction were preincubated for 2 h prior to crystallization, multiple dissociation and reassociation events must have taken place before the crystals formed.

(ii) pol β is a specialized DNA polymerase whose natural substrate is gapped duplex DNA. Several recent biochemical reports suggest that pol β is able to completely fill short single-stranded gaps, such as those occurring as intermediates in DNA excision repair, via a mechanism that strictly requires a 5′ phosphate at the downstream boundary of the gap (24, 27, 31). pol β is expected to have two binding sites for double-stranded DNA (one to bind the upstream template-primer duplex and the second to bind the downstream duplex), both of which would be occupied during the late stages of the gap-filling reaction.

(iii) Although the 8-kDa positively charged N-terminal domain of pol β does not bind the DNA template-primer in either of the reported crystal structures, it has been implicated in binding DNA substrates. For example, it has been reported that phosphorylation by protein kinase C of two serine residues located in the 8-kDa domain dramatically decreases pol β activity (29). It has also been shown that the 8-kDa domain is the site of an activity that catalyzes the release of 5′-terminal deoxyribose phosphate residues from incised apurinic-apyrimidinic sites during base excision repair (18).

When a model was prepared of pol β in a complex with DNA in which the orientation of the DNA was the same as that seen in the HIV-1 RT DNA crystals, only a relatively minor rotation of the flexible 8-kDa domain of pol β was necessary to bring it into extensive contact with the double-stranded portion of the template-primer. These molecular modeling experiments may have bearing on the interaction between pol β and one of its natural substrates. With some rearrangements, it is possible to include the DNA from the pol β structure in the model and to derive a structure in which pol β has bound what is essentially its appropriate substrate: a gapped duplex (Fig. 1). It is not clear that positioning the additional portion of the gapped DNA duplex based on the HIV-1 RT DNA coordinates is entirely appropriate. However, the template-primer in the Pelletier et al. structure cannot be in the position that a gapped duplex would occupy. Examination of Fig. 4 and 6 of Pelletier et al. (22) shows that a gapped duplex cannot be accommodated in this structure: the terminus of the template-primer lies directly against helix M (see also Fig. 1). We suggest it should be possible to accommodate a DNA duplex that would correspond to the upstream portion of a gapped duplex substrate in approximately the same position relative to the palm subdomains seen in other polymerase structures.

Obviously, the most physiologically relevant structure of pol β would be that of a complex with the natural substrate, a gapped DNA duplex. Analysis of this type of structure should resolve the discrepancies discussed above. However, a biochemical approach can also be taken. For example, if the ternary complex of pol β, the 11 by 7-nucleotide fragment, and ddCTP represents a reaction intermediate as proposed by Pelletier et al. (22), the absence of interactions between the short template and the DNA-binding 8-kDa domain suggests that the readily crystallizable 31-kDa form of pol β (which lacks the 8-kDa domain) and the full-size 39-kDa form of pol β should be able to extend a template-primer with a short template overhang with comparable efficiency. The large difference in activity of the 31- and 39-kDa forms of pol β should be manifested only with template-primers that have relatively long template extensions. Such an experiment would be analogous to the analysis of the RT-DNA complex mentioned earlier, in which differences between drug-resistant and sensitive forms of RT could be demonstrated only with template extensions long enough to reach the mutated site (6).

In conclusion, while questions remain about the biological relevance of the pol β-DNA complexes, there can be little doubt that the HIV-1 RT-DNA complex is a biologically relevant structure. Moreover, the similarity of the palm subdomains of HIV-1 RT, MLV RT, Klenow fragment, *Taq* polymerase, and T7 RNA polymerase provides a strong argument that these polymerases all bind DNA substrates in the same orientation.

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REFERENCES

1. **Abbotts, J. D. N. SenGupta, B. Smudzka, S. G. Widen, V. Notario, and S. H. Wilson.** 1988. Expression of human DNA polymerase beta in *Escherichia coli* and characterization of the recombinant enzyme. Biochemistry **27:**901–909.
2. **Arnold, E., A. Jacobo-Molina, R. G. Nanni, R. L. Williams, X. Lu, J. Ding, A. D. Clark, Jr., A. Zhang, A. L. Ferris, P. Clark, A. Hizi, and S. H. Hughes.** 1992. Structure of HIV-1 reverse transcriptase/DNA complex at 7 Å resolution showing active site locations. Nature (London) **357:**85–89.
3. **Boyer, P. L., A. L. Ferris, and S. H. Hughes.** 1992. Mutational analysis of the fingers domain of human immunodeficiency virus type 1 reverse transcriptase. J. Virol. **66:**7533–7537.
4. **Boyer, P. L., A. L. Ferris, and S. H. Hughes.** 1992. Cassette mutagenesis of the reverse transcriptase of human immunodeficiency virus type 1. J. Virol. **66:**1031–1039.

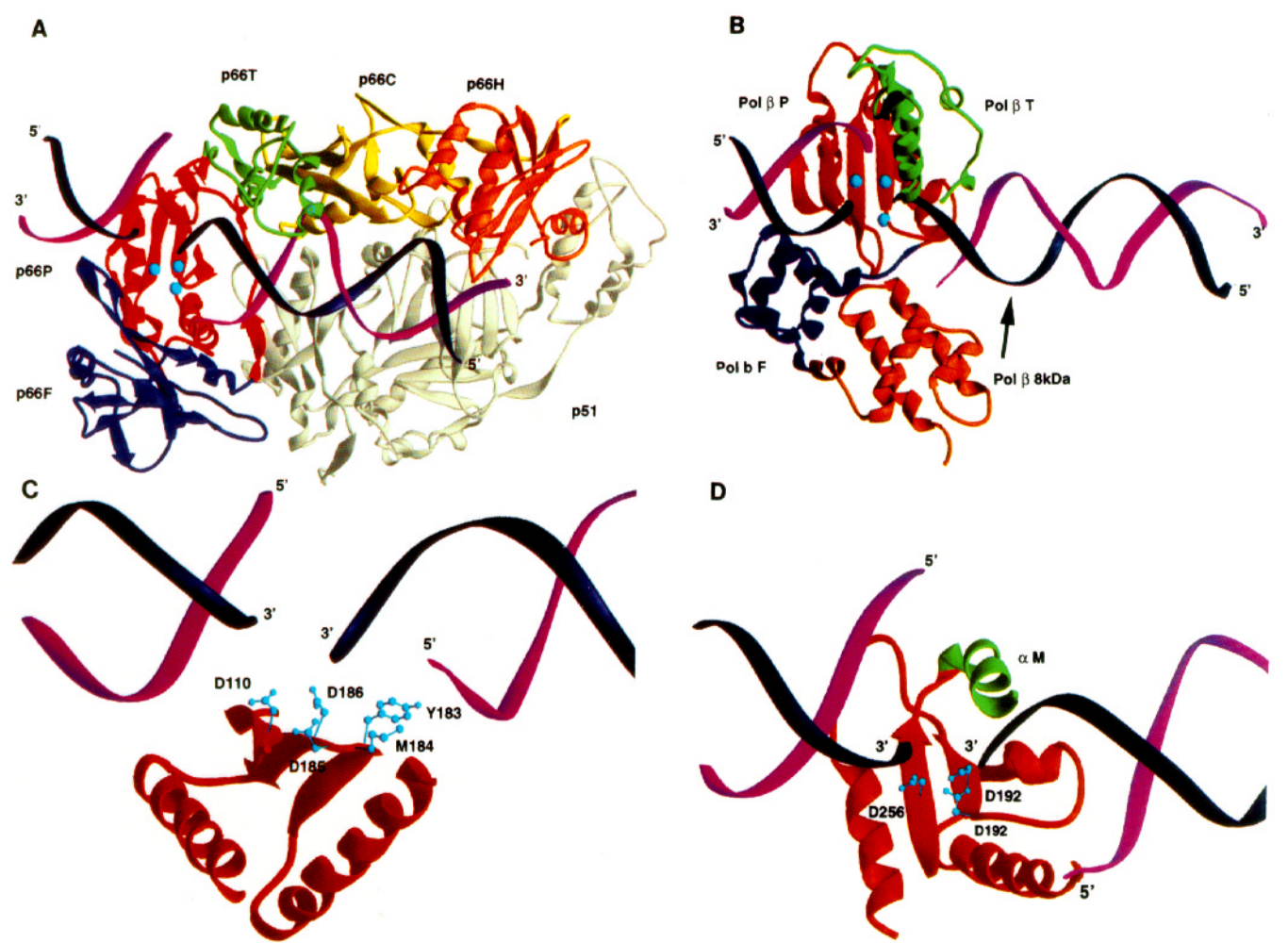
4a. **Boyer, P. L., and S. H. Hughes.** Unpublished observations.

5. **Boyer, P. L., C. Tantillo, A. L. Ferris, E. Arnold, and S. H. Hughes.** 1994. Mutational analysis of the fingers and palm subdomains of human immunodeficiency virus type-1 (HIV-1) reverse transcriptase. J. Mol. Biol. **243:**472–483.
6. **Boyer, P. L., C. Tantillo, A. Jacobo-Molina, R. G. Nanni, J. Ding, E. Arnold, and S. H. Hughes.** 1994. Sensitivity of wild-type human immunodeficiency virus type 1 reverse transcriptase to dideoxynucleotides depends on template length; the sensitivity of drug-resistant mutants does not. Proc. Natl. Acad. Sci. USA **91:**4882–4886.
7. **Davies, J. F., R. J. Almassy, Z. Hostomska, R. A. Ferre, and Z. Hostomsky.** 1994. 2.3 Å crystal structure of the catalytic domain of DNA polymerase β. Cell **76:**1123–1133.
8. **Delarue, M., O. Poch, N. Tordo, D. Moras, and P. Argos.** 1990. An attempt to unify the structure of polymerases. Protein Eng. **6:**461–467.

8a. **Ding, J., and E. Arnold.** Unpublished observations.

9. **Furfine, E. S., and J. E. Reardon.** 1991. Reverse transcriptase RNase H from the human immunodeficiency virus. Relationship of the DNA polymerase and RNA hydrolysis activities. J. Biol. Chem. **266:**406–412.
10. **Georgiadis, M. M., S. M. Jessen, C. M. Ogata, A. Telesnitsky, S. P. Goff, and W. A. Hendrickson.** 1995. Mechanistic implications from the structure of a catalytic fragment of Moloney murine leukemia virus reverse transcriptase. Structure **3:**879–892.
11. **Ghosh, M., K. J. Howard, C. E. Cameron, S. J. Benkovic, S. H. Hughes, and S. F. J. Le Grice.** 1995. Truncating α-helix E′ of p66 human immunodeficiency virus reverse transcriptase modulates RNase H function and impairs DNA strand transfer. J. Biol. Chem. **270:**7068–7076.
12. **Gopalakrishnan, V., J. A. Peliska, and S. J. Benkovic.** 1992. Human immunodeficiency virus type 1 reverse transcriptase: Spatial and temporal relationship between the polymerase and RNase H activities. Proc. Natl. Acad. Sci. USA **89:**10763–10767.

13. **Holm, L., and C. Sander.** 1995. DNA polymerase β belongs to an ancient nucleotidyltransferase superfamily. Trends Biochem. Sci. **20:**345–347.
14. **Jacobo-Molina, A., J. Ding, R. G. Nanni, A. D. Clark, Jr., X. Lu, C. Tantillo, R. L. Williams, G. Kamer, A. L. Ferris, P. Clark, A. Hizi, S. H. Hughes, and E. Arnold.** 1993. Crystal structure of human immunodeficiency virus type 1 reverse transcriptase complexed with double-stranded DNA at 3.0 Å resolution shows bent DNA. Proc. Natl. Acad. Sci. USA **90:**6320–6324.
15. **Kim, Y., S. H. Eom, J. Wang, D.-S. Lee, S. W. Suh, and T. A. Steitz.** 1995. Crystal structure of Thermus aquaticus DNA polymerase. Nature (London) **376:**612–616.
16. **Kohlstaedt, L. A., J. Wang, J. M. Friedman, P. A. Rice, and T. A. Steitz.** 1992. Crystal structure at 3.5 Å resolution of HIV-1 reverse transcriptase complexed with an inhibitor. Science **256:**1783–1790.
17. **Korolev, S., M. Nayal, W. M. Barnes, E. Di Cera, and G. Waksman.** 1995. Crystal structure of the large fragment of *Thermus aquaticus* DNA polymerase I at 2.5-Å resolution: structural basis for thermostability. Proc. Natl. Acad. Sci. USA **92:**9264–9268.
18. **Matsumoto, Y., and K. Kim.** 1995. Excision of deoxyribose phosphate residues by DNA polymerase β during DNA repair. Science **268:**699–702.
19. **Metzger, W., T. Hermann, O. Schatz, S. F. J. Le Grice, and H. Heumann.** 1993. Hydroxyl radical footprint analysis of human immunodeficiency virus reverse transcriptase-template · primer complexes. Proc. Natl. Acad. Sci. USA **90:**5909–5913.
20. **Ollis, D. L., P. Brick, R. Hamlin, N. G. Xuong, and T. A. Steitz.** 1985. Structure of large fragment of *Escherichia coli* DNA polymerase I complexed with dTMP. Nature (London) **313:**762–766.
21. **Patel, P. H., A. Jacobo-Molina, J. Ding, C. Tantillo, A. D. Clark, R. Raag, R. G. Nanni, S. H. Hughes, and E. Arnold.** 1995. Insights into DNA polymerization mechanisms from structure and function analysis of HIV-1 reverse transcriptase. Biochemistry **34:**5351–5363.
22. **Pelletier, H., M. R. Sawaya, A. Kumar, S. H. Wilson, and J. Kraut.** 1994. Structures of ternary complexes of rat DNA polymerase β, a DNA template-primer, and ddCTP. Science **264:**1891–1903.
23. **Poch, O., I. Sauvaget, M. Delarue, and N. Tordo.** 1989. Identification of four conserved motifs among the RNA-dependent polymerase encoding elements. EMBO J. **8:**3867–3874.
24. **Prasad, R., W. A. Beard, and S. H. Wilson.** 1994. Studies of gapped DNA substrate binding by mammalian DNA polymerase β. J. Biol. Chem. **269:** 18096–18101.
25. **Rausch, J. S., B. M. Wöhrl, and S. F. J. Le Grice.** J. Mol. Biol., in press.
26. **Sawaya, M. R., H. Pelletier, A. Kumar, S. Wilson, and J. Kraut.** 1994. Crystal structure of rat DNA polymerase β: evidence for common polymerase mechanism. Science **264:**1930–1935.
27. **Singhal, R. K., and S. H. Wilson.** 1993. Short gap-filling synthesis by DNA polymerase β is processive. J. Biol. Chem. **268:**15906–15911.
28. **Steitz, T. A., S. J. Smerdon, J. Jäger, and C. M. Joyce.** 1994. A unified polymerase mechanism for nonhomologous DNA and RNA polymerases. Science **266:**2022–2025.
29. **Tokui, T., M. Inagaki, K. Nishizawa, R. Yatani, M. Kusagawa, K. Ajiro, Y. Nishimoto, T. Date, and A. Matsukage.** 1991. Inactivation of DNA polymerase β by *in vitro* phosphorylation with protein kinase C. J. Biol. Chem. **266:**10820–10824.
30. **Unge, T., S. Knight, R. Bhikhabhai, S. Lovgren, Z. Dauter, K. Wilson, and B. Strandberg.** 1994. 2.2 Å resolution structure of the amino-terminal half of HIV-1 reverse transcriptase (fingers and palm subdomains). Structure **2:**953–961.
31. **Wiebauer, K., and J. Jiricny.** 1990. Mismatch-specific thymine DNA glycosylase and DNA polymerase beta mediate the correction of G · T mispairs in nuclear extracts from human cells. Proc. Natl. Acad. Sci. USA **87:**5842–5845.
32. **Wörhl, B. M., M. M. Georgiadis, A. Telesnitsky, W. A. Hendrickson, and S. F. J. Le Grice.** 1995. Footprint analysis of replicating murine leukemia virus reverse transcriptase. Science **267:**96–99.
33. **Wörhl, B. M., and K. M. Moelling.** 1990. Interactions of HIV-1 ribonuclease H with polypurine tract containing RNA/DNA hybrids. Biochemistry **29:** 10141–10147.
34. **Wöhrl, B. M., C. Tantillo, E. Arnold, and S. F. J. Le Grice.** 1995. An expanded model of replicating human immunodeficiency virus reverse transcriptase. Biochemistry **34:**5343–5350.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