extent of protection by KF of DNA upstream of the primer terminus (*24*) is more consistent with the model deduced from the co-crystal (*2*) than with the earlier model (*4*).

Thus, we conclude that all four of the polymerases of known structure will be able to use the same two-metal-ion mechanism of catalysis in spite of the detailed differences in the way that these metal ions are anchored to the protein in each of the four polymerases. While the 3′-OH of the primer terminus, the phosphates of dNTP (or ribonucleoside triphosphate), and the two-metal ions should show the same relative orientations in all polymerases, the precise structure of the product duplex as it emerges from the site of synthesis may well differ. High-resolution crystal structures of other polymerases with primer-template and dNTP [like the ternary complex of pol β reported by Pelletier *et al.* (*1*)] will be necessary to allow more precise relative alignments of polymerases and detailed mechanistic comparisons to be made.

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Polymerase Structures and Mechanism

The comment by T. Steitz *et al.* (*1*) disagrees with the conclusions in our paper (*2*) that were based on our structural studies of rat DNA polymerase β (*2, 3*). Nevertheless, given the current state of the literature on DNA and RNA polymerases, every proposal presented in our paper is reasonable and can be tested experimentally (*2*).

The proposals of Steitz *et al.* (*1*) depend on the idea that pol β is nonhomologous with other polymerases (that is, that pol β arose from a different ancestor), and Steitz *et al.* (*1*) cite our report (*3*) as taking the view that the palm subdomain of pol β is a result of convergent evolution. However, our discussions (*3*) favor neither convergence nor divergence because there appears to be equally strong evidence supporting both sides of the debate over the evolution of pol β.

In contrast to the effect it has on the proposals of Steitz *et al.* (*1*), the evolutionary history of pol β has no bearing on our structural alignments and proposals (*2, 3*). Regardless of whether pol β converged or diverged, there are three catalytically important, highly conserved carboxylic acid residues located in the palm subdomain, and only one unambiguous orientation of pol β superposes these residues with those of all other polymerase crystal structures (*2–7*) (Fig. 1). [Even in the case of covergent evolution (*8*), it is expected that the catalytic residues, at the very least, superpose. Otherwise, it is not really convergent evolution.] That the rest of the palm subdomain of pol β then happened to superpose well with the other polymerase palms was additional evidence that the structural alignments (*3*) were correct.

Instead of relying on structural alignments of conserved active site residues, Steitz *et al.* (*1*) suggest that a greater weight be placed on functional alignments of nonhomologous entities in the fingers and thumb subdomains of the polymerase structures. However, a "functionality" approach can still favor our current pol β structural alignments (*2, 3*). The thumb subdomain of pol β, for instance, shows greater flexibility than the fingers subdomain (*2*), as is also the case for other polymerases (*5, 6*). In addition, structural elements that are similar to alpha helices M and N of pol β only in that they protrude into the active site from the same "thumb" side of the DNA binding channel are present in all other polymerase structures and are proposed to

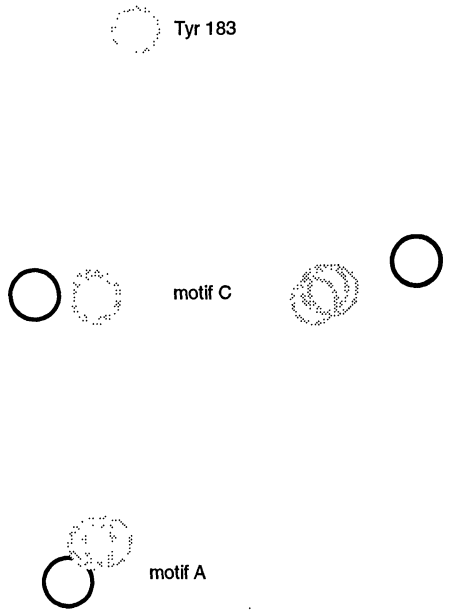

Fig. 1. Least squares superposition of the Cα's for the catalytically critical carboxylate residues of four different polymerase active sites (*2–7*). Dark circles represent Asp^{190} and Asp^{192} (motif C), as well as Asp^{256} (motif A), of pol β, and lighter circles represent active site carboxylates of the other three polymerases for which crystal structures are known [see (*3*, figure 2) for a listing of the residue numbers]. The 3′-OH of the DNA primer terminus approaches the active site from the direction of the bottom of the page (*2*). In keeping with previous proposals (*2*), the Cα position of Tyr^{183} of RT is included as an integral part of the active site for that polymerase and allows the 3′-OH of the DNA primer terminus to approach the active site from the direction of the top of the page, as is the case when RT is in an anti-pol β mode of DNA binding (*2*). The protein side chains [not shown (*14*)], extend out of the plane of the page toward the viewer.

participate in the same function, namely, nucleotide selectivity (*2*).

The functional alignments of Steitz *et al.* (*1*) require that their proposed common polymerase active site exclude all protein side chains and consist of only two divalent metal ions. One implication of their idea is that the coordination states of the metal ions are inconsequential. This contradicts the observation that the pol β active site is highly asymmetric with respect to the two metal ions (*2*) and that only one metal ion, not two, binds to pol β in the absence of nucleotide substrate (*9*). Although the participation of protein side chains is probably not an absolute requirement for all nucleotidyl transfer mechanisms (*2*), it is evident that no matter what the catalytic scaffolding, something must support two divalent metal ions that differ significantly in binding affinities, geometries, and functions. The arrangement of the carboxylic acid residues in the polymerase active site is therefore important and should not be readily dismissed.

Many arguments have been presented in favor of the physiological relevance of other polymerase-DNA crystals (*1*, *5*, *6*), but they do not appear to be convincing. The direction of DNA-binding proposed by Steitz *et al.* (*1*) is not in accord with the nick translation activity of *Escherichia coli* DNA pol I (*2*). In addition, it is not possible to model a reasonable nucleotidyl transfer mechanism, similar to that described for pol β (*2*), in the RT active site with the DNA template-primer of the RT-DNA crystal structure (*5*) fixed in its present position.

The catalytic mechanism proposed by Steitz *et al.* for the nucleotidyl transfer reaction (*1*), although somewhat altered from their previously proposed three-metal-ion mechanism (*10*, *11*), differs significantly from the one described in our paper (*2*). Contrary to statements made by Steitz *et al.* (*1*), neither of the two metal ions in the pol β ternary complex structure (*2*) "interacts with" all three phosphates of the nucleotide (it is assumed that "interacts with" means "coordinates"). This type of interaction has been reported only for the structure of the fragmented pol β enzyme in the absence of DNA (*12*). Because the structures reported in (*12*) are not available from the Brookhaven Protein Data Bank, no further comment can be made about this point.

Steitz *et al.* state that it is not possible for Asp^{256} of pol β to act both as a metal ion ligand and a general base, as proposed in our paper (*2*). They base their argument on the position of Asp^{256} in the ternary complex structures, as well as on chemical considerations. With regard to the side chain position of Asp^{256}, the 3′-OH group is clearly absent from the dideoxycytidine primer terminus in the pol β ternary complex structures (a necessary condition in order to obtain these crystals), and as suggested [figure 6 in (*2*)], the side chain position of Asp^{256} probably differs when the 3′-OH is present. Therefore, the arguments of Steitz *et al.* (*1*) concerning the unfavorable distance and geometry of the proton acceptor most likely become inapplicable during catalysis, when all the reactive groups are present in the active site.

As for chemical considerations, although the pKa of Asp^{256} must be affected by its coordination to a divalent metal ion, it is not necessarily true that because of this, it is not possible for Asp^{256} to be the hydrogen bond acceptor in the pol β active site. What should not be overlooked is that the pKa of the hydrogen bond donor (the 3′-OH of the primer terminus) is probably also drastically affected by the same metal ion [figure 6 in (*2*)]. In fact, one feature that makes the proposed transition state (*2*) particularly appealing is that the pKa of both the donor and the acceptor group can be regulated by a single metal ion; relatively small changes in metal-oxygen coordination geometry, which in turn translate into large changes in pKa values for the donor-acceptor pair, can be manipulated by the pol β molecule to fine tune catalysis.

Although Steitz *et al.* (*1*) take issue with the proposed role of Asp^{256} in the nucleotidyl transfer reaction of pol β (*2*), they have assigned the same role (both a metal ligand and a proton acceptor) to Glu^{357} in the catalytic mechanism of the 3′ → 5′ exonuclease of *E. coli* DNA pol I (*13*). Nevertheless, the possibility that some other entity, such as a water molecule, acts as the proton acceptor in the nucleotidyl transfer reaction should not be ruled out. In fact, this idea is consistent with the proposal in our paper that a tyrosine residue (Tyr^{183} of HIV-1 RT) can play the same role in catalysis as pol β's Asp^{256}, that is, when RT is in an anti-pol β mode of template-primer binding (*2*) (Fig. 1).

Although the interpretations of the data from our structural studies of pol β contradict interpretations of polymerase data by other research groups, it does not follow that the proposal of Steitz *et al.* (*1*) is the best solution to this conundrum. We eagerly await further polymerase-DNA x-ray structures, preferably determined from crystals grown at physiological ionic strength, so that debate over DNA binding and directionality, as well as debate over the mechanism of nucleotidyl transfer, can be superseded by discussions concerning yet unresolved mysteries, such as the fine structural details governing polymerase fidelity and processivity.

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9. As pointed out in a "Note added in proof" (*3*), the observation that only one metal ion binds in the active site of pol β in the absence of nucleotide substrate [reference 65 in (*2*); reference 28 in (*3*)] is in disagreement with observations reported by another group (*12*). We have not observed metal-ion-binding behavior described in (*12*), despite repeated efforts to reproduce the conditions exactly (H. Pelletier and M. R. Sawaya, unpublished data). Although Steitz *et al.* (*1*) state that the metal-ion-binding behavior of the Klenow fragment differs from that observed for pol β, we are not able to comment on this point because the metal-ion-binding data for the Klenow fragment are not published.
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14. Data for side chain positions for the RT-DNA structure (*5*), the KF-DNA structure (*6*), and the T7 RNA polymerase structure (*7*) are not available at present. Note that side chain positions may change upon complex formation with DNA and nucleotide substrates, as has been observed in our structural studies with pol β (*2*, *3*).
15. I thank M. R. Sawaya for aid in preparing Fig. 1, J. Kraut for reviewing the manuscript, and both for useful discussions.

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