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addenda and errata

Retraction of articles by H. M. Krishna Murthy *et al.*

Two papers by H. M. Krishna Murthy *et al.* are retracted.

Two papers by H. M. Krishna Murthy *et al.* (Krishna Murthy *et al.*, 1999; Urs *et al.*, 1999) are retracted by the journal. This follows investigation by the University of Alabama at Birmingham, Alabama, USA, of structures deposited by H. M. Krishna Murthy. Krishna Murthy has noted that he is not in agreement with the retractions.

References

Krishna Murthy, H. M., Judge, K., DeLucas, L., Clum, S. & Padmanabhan, R. (1999). *Acta Cryst.* D**55**, 1370–1372.
Urs, U. K., Murali, R. & Krishna Murthy, H. M. (1999). *Acta Cryst.* D**55**, 1971–1977.

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tructure of a poly erase sho s a ne orientation for the structure speci c nuclease do ain

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B Reference aq DNA polymerase, 1cm .

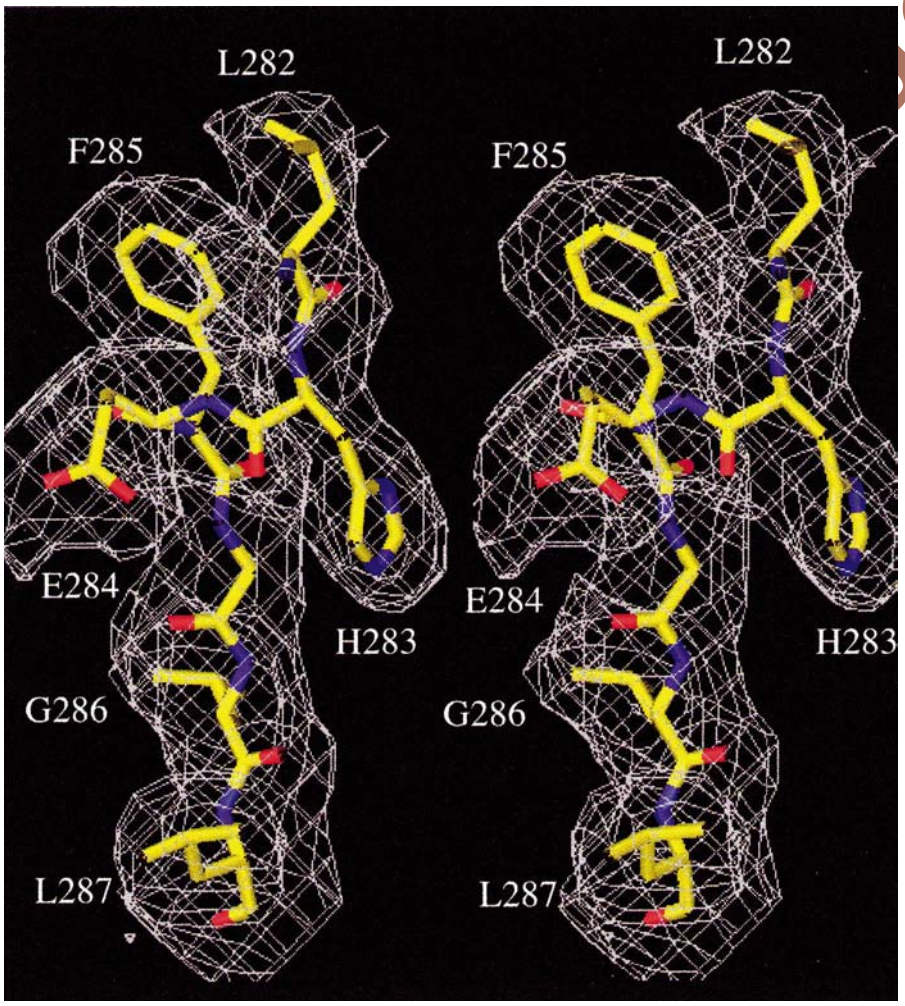
er s a at c s D A polymerase consists of the polymerase, the structure speci c nuclease and the vestigial editing nuclease domains. Three dimensional structures of the native en yme and its comple with D A have already been reported. The structure of a comple with an inhibitory antibody has also been determined. The structure of the native en yme in a different crystal form determined at 2. A is reported here. ptimi ed anomalous diffraction measure ments made at the holmium edge were valuable in validating solutions obtained through molecular replacement. The structure of the polymerase domain is similar to those reported previously, while the relative orientation of the structure speci c nuclease domain is signi cantly different from those of the native en yme and the D A comple ; it is, however, identical to that observed in the structure of the ab comple . n the structures of the native en yme and of the D A comple reported previously, the active site of the structure speci c nuclease domain is too far from that of the polymerase domain, ma ing it dif cult to propose a structural model for the primer e cision and nic translation activities of the en yme. n the present structure, the two active sites are considerably closer. Ta en together, the reported structure of the native en yme, that of the ab comple and the present structure imply that the different orientation of the structure speci c nuclease domain is probably a conse uence of intrinsically high relative mobility between these two domains in this en yme.

. Introduction

D A polymerase from *. a at c s* (Ta P) is of considerable biological, technological and economic importance. Li e D A polymerases from other organisms (Marians, 1992), it is the central participant in replication of genetic information with great delity; unli e similar en ymes from mesophilic organisms, it carries out this activity at elevated temperatures (Chien *et al.*, 197 ; Kaledin *et al.*, 1979; Lawyer *et al.*, 19 9). Ta P is also used widely in carrying out polymerase chain reaction (PCR) e periments (Arnheim & rlich, 1992). PCR plays a central role in the technology of molecular biology and is also employed in clinical diagnostics (right & ynford Thomas, 1990) and in D A based forensic analyses (Decorte & Cassiman, 1993).

The domain composition of Ta P is similar to that of its *sc er c a c l* analog and consists of a polymerase (pol, residues 2 – 32), a structure speci c ′–3′ nuclease (nuc, residues 1–290) and a third domain (residues 291– 23) that is the structural analog of the editing 3′– ′ nuclease domain of the . *c l* en yme (Kim *et al.*, 199 ; Korolev *et al.*, 199).

However, the latter nuclease domain in Ta P (v edit) is vestigial and has little se uence similarity to its . c l coun terpart. n particular, the residues at the active site, Asp3 , lu3 7, Asp 2 and lu 01, in . c l are replaced in Ta P by ly30 , al310, Leu3 and Arg 0 , respectively. These residues in Ta P are incapable of binding the metal ions necessary for nuclease activity and v edit does not function as an editing nuclease (Kim *et al.*, 199). The nuc domain is used by the en yme for primer e cision and nic translation activ ities (Lyamichev *et al.*, 1993). The structures of both the native en yme (Kim *et al.*, 199) and that of a comple with D A have been reported (om *et al.*, 199). n addition, the structure of the en yme without the nuc domain (Korolev *et al.*, 199) and several comple es with D A and other substrates have been determined (Li, Kong *et al.*, 199 ; Li, Korolev *et al.*, 199). Crystalli ation of the en yme in a different crystalline form as well as the structure of its comple with an inhibitory ab have also been reported by us (Urs *et al.*, 199 ; Murali *et al.*, 199). The reported structures show that the structure of the pol domain of Ta P is very similar to that of the . c l en yme, while that of the v edit domain is signi cantly different from that of its . c l analog. The structure of native Ta P shows (Kim *et al.*, 199) that there are three metal ions at the active site of the nuc domain, two Mn^{2} and a n^{2} , which is consistent with the two metal ion mechanism that has been proposed for nuclease activity (Beese & Steit , 1991). The active site of the pol domain in the native structure is appro imately 70 A from that of the nuc domain. However, current biochemical models (Lyamichev *et al.*, 1993) for nic translation by Ta P postulate closer spatial ju taposition of the two domains, ma ing it dif cult to suggest a structural model for nic translation and primer e cision by Ta P. Measurements of the radius of gyration of Ta P in solution also imply a more compact association of the pol and nuc domains (Kim *et al.*, 199). The structure of the native en yme in a new crystal form reported here shows a drama tically different relative orientation of the nuc domain with respect to the pol domain. The distance between the pol and nuc active sites in this structure is appro imately 0 A, a value which is closer to that found between the editing nuclease and pol domain active sites in the . c l en yme. However, this different orientation is still not consistent with biochemical models of primer e ci sion and nic translation (Lyamichev *et al.*, 1993; Lyamichev, . & Dahlberg, J., personal communication). t does suggest, however, that there is a remar able degree of e ibility in orientation between these two domains in Ta P. The relative orientation seen in this structure is identical to that seen in the comple of the en yme with an ab (Murali *et al.*, 199) and indicates that the orientation in that structure is probably not a conse uence of en yme– ab inter action.



igure
A stereo drawing of the t of the atomic model to the electron density. A 2 – $_{c}$ map contoured at 1σ is shown with the nal re ned model superimposed. C atoms are coloured yellow, atoms red and atoms blue. Residues 2 2–2 7 in the connector region between the pol and nuc domains are shown. Phases for the map were calculated after omission of residues 2 2–2 7 and restrained re nement of the rest of the structure for several cycles. Some residues are labeled.

2. Materials and ethods

2. . Crystal treat ent data easure ent and processing

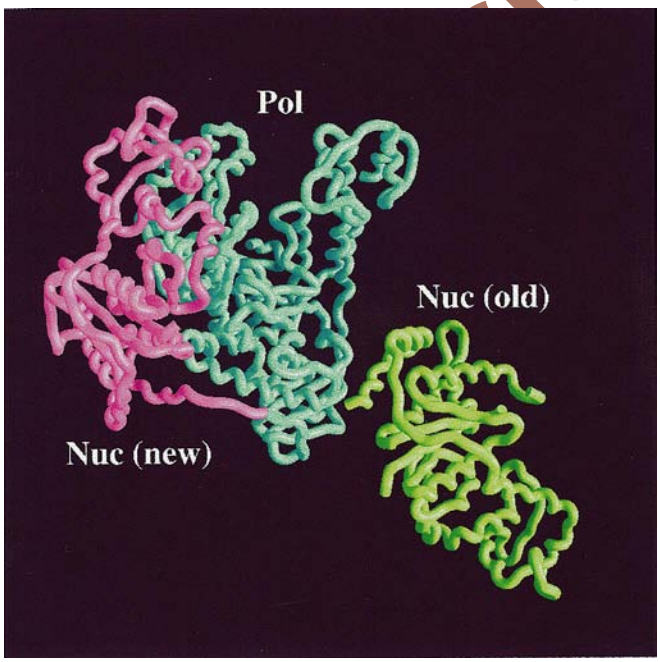
Crystalli ation of Ta P in a cubic space group (23 or 2_13, *a* 193.) and measurement of native data to A resolution have been reported (Urs *et al.*, 199). ative data were e tended to 2. A using larger crystals and measuring data on the A1 station at CH SS. The data measurement was carried out at 291 K using a CCD detector. Processing was performed using and scaling was performed in *CA AC* (twinows i & Minor, 1997). Statistics

able
Data measurement and structure re nement.

Resolution (A)	bserved	sym	⟨ σ( )⟩¶	⟨Redundancy⟩	cryst	free
.20	. (100)	0.03	1 .3	7.1	0.193	0.2 2
.13	.1 (100)	0.031	1 .	.7	0.211	0.2
3. 1	3.1 (97)	0.0	1 .7	.9	0.17	0.2 7
3.2	3.9 (9)	0.0	1 .3	7.2	0.17	0.2
3.0	3.9 (93)	0.0 7	12.	.3	0.1	0.2 0
2.	. (97)	0.077	12.9	.7	0.179	0.2 2
2.72	.1 (9)	0.0	13.9	3.9	0.19	0.277
2. 0	3. (9)	0.0 1	12.	3.9	0.211	0.2
All	. (9)	0.0	21 .2	.3	0.192	0.2

Resolution; lowest measured re ections were at 30 A. Percentage of measured re ections with $2\sigma(\ )$ (completeness in parentheses). $\sum(|\ -\langle\ \rangle|)\ \sum$. ¶ Average signal to noise ratio. Average number of measurements per re ection. $\sum(|\ _{obs} - \ _{cal}|)\ \sum\ _{obs}$. $\sum(|\ _{obs} - \ _{cal}|)\ \sum\ _{obs}$ for 10 (31) of re ections chosen at random throughout the resolution range.

on the native data set, constructed using si crystals, are listed in Table 1. ndogenous metal ions bound to the native en yme were replaced by se uential soa ing of crystals. irst, crystals were soa ed in buffer (100 m Tris–HCl pH .3, 0 satu rated ammonium sulfate) containing 0. glutaraldehyde for 10 min. Subse uent soa ing for h in buffer containing 0. m phenanthroline and 3 m DTA removed all replaceable metal ions. The crystals were nally soa ed in buffer containing 1 m $HoCl_3$ overnight to populate the metal binding sites with Ho^3 ions. Diffraction data were recorded on a CCD detector using cryogenic techni ues on the 2 station at CH SS. The wavelength for data measure ment, after a preliminary uorescence scan, was set at 1. 29 3 A in order to ma imi e the measured Bijvoet signal.



igure 2
ew position of the nuc domain. C^{α} traces of the pol domain (cyan), including the v edit domain, position of the nuc domain in this structure and that of the ab comple (magenta) and that of the nuc domain in other structures of Ta P (green) are shown.

A total of four metal ions are e pected to be bound speci cally to the native en yme, two (Mg^2) at the pol active site and three (2 Mn^2 and n^2) at the nuc active site. ne of the two ions at the pol active site is stabili ed by ligation through phosphate groups of the incoming nucleotide (Sawaya *et al.*, 1997; Doublie *et al.*, 199 ; Huang *et al.*, 199 ; Li, Korolev *et al.*, 199); thus, in the native state, only one Mg^2 ion is bound to the pol active site. Assuming replacement by Ho^3 of all four ions and an $''$ value of 12.9 for Ho^3 at 1. 29 3 A, a Bijvoet signal of . is e pected at ero scattering angle (Hendric son, 1991; Krishna Murthy, 199 ; Krishna Murthy *et al.*, 19). Because of the stresses that the crystals were inevitably subjected to during the soa ing protocol, measurable diffraction was limited to A, although no signi cant changes in unit cell parameters were observed. Data were, as before, processed and scaled in and *CA AC* , respectively (twinows i & Minor, 1997), eeping Bijvoet mates unmerged. Statistics are presented in Table 2.

2.2. tructure solution and re ne ent

The structure was determined using molecular replacement (MR) supplemented by information from the optimi ed anomalous diffraction measurements. ative data in the resolution range – A and with $3\sigma(\ )$ were used in MR calculations. Coordinates of the pol and v edit domains from reported structures (Kim *et al.*, 199 ; Korolev *et al.*, 199) were used together as a search model in *A e* (ava a, 199), as implemented in the *CC* suite (Collaborative Computational Project, umber , 1993), in order to determine their orien tation in the cubic cell. A correlation coef cient (CC) of 10. obtained for the highest solution (ne t highest CC, .) uni uely determined the orientation. A translation function calculation for the highest 20 rotation solutions, performed in *A e*, gave a uni ue pea for the highest rotation solution, with a CC of 20.2, clearly offset from the ne t highest, which had a CC of 9. . The value for the best translation solution was 0. 23, with that for the ne t best solution being 0. 1. Rigid body tting in *A e* improved the CC and value for the best solution to 31. and 0. 01, respectively. The transla tion function calculations also served to establish the space group as 23 rather than 2_13, as e uivalent calculations in the latter space group did not produce interpretable results. ncorporating the nuc domain in the same orientation as that seen in the reported native structure, using a rigid body transformation based on the C^{α} coordinates of respective pol domains, led to numerous close contacts between C^{α} atoms of the nuc domain and symmetry related copies of the nuc and pol domains. These severe pac ing clashes implied that the orientation of the nuc domain in our structure of Ta P was substantially different from that of the reported structure.

A e again yielded a good rotation solution (CC 1 .2, ne t highest .) for the nuc domain orientation when co

able 2
Bijvoet differences statistics.

Resolution (A)	Re ections	$_{Bij}$ (centric)	$_{sym}$	⟨ σ( )⟩¶
.7	9 (92)	0.07 (0.021)	0.023	2 .3
.	7 (79)	0.110 (0.037)	0.0	1 .7
.0	7 (70)	0.1 (0.07)	0.0	. 0
All	1 (77)	0.093 (0.0 7)	0.072	12.

Percentage of re ections with 3σ( ) (centric values in parentheses). $\sum(|^{+} - ^{-}|)$ 0. $\sum(^{+} + ^{-})$; values for centric re ections are in parentheses. $\sum(|\ - \langle\ \rangle|)$ $\sum$. ¶ Average signal to noise ratio.

able
Bijvoet differences Patterson map statistics.

		Signal to noise ratio		
Site	bserved vectors	Ma	Min	Average
A–*A*	93	.	.7	.1
–		.	3.9	3.7
C–*C*	92	7.	3.1	3.9
A–		.	3.0	3.
A–*C*	9	.	2.	2.9
–*C*		.7	2.3	3.1
All		7.	2.3	2.7

See te t for site designation. Percentage of theoretical number. perimental pea s were de ned as observed if they were at least two times the r.m.s. density of the map. Ratio of pea height to r.m.s. density of map. or purposes of comparison, the value of signal to noise ratio for the origin pea was 3 . .

ordinates of the nuc domain alone from the reported structure (Kim *et al.*, 199) were used as the search model in cross rotation function calculations. However, many attempts to obtain a translation function solution in *A* *e* both in the presence and absence of the ed pol domain did not succeed. The highest 20 solutions from the rotation calculations were then used in phased translation function calculations performed in *C* (Tic le, 1992), a part of the *CC* pac age (Collaborative Computational Project, umber , 199). Both the T2 and the T functions implemented in *C* gave similar results, with an r.m.s. deviation between them of 1. A, for the translation component for the nuc domain. This solu tion was well separated from the ne t highest solution, with the signal to noise ratio for the highest pea in the T2 function being . and that for the ne t highest pea being 1. . Similar results were obtained using the phased translation function implemented in (Brunger, 1992). hen placed in this orientation in the 23 cell, the nuc domain did not yield any main chain contacts with symmetry mates that were less than 2.7 A. Because of the dif culties encountered in using *A* *e* to place the nuc domain in the 23 cell and the dif culty in assigning molecular partners unambiguously when MR calculations are carried out with fragmented search models, it was decided to obtain independent con rmation from metal substitution e periments.

Data from the Ho^{3} substituted crystals were scaled using local scaling routines from *A* (Hendric son, 1991) in order to obtain an acceptable set of Bijvoet differences; statistics are listed in Table 2. *CC* routines were used in calculating a Bijvoet differences Patterson map with a set of 71 non centric re ections. A trial set of coordinates for the Ho^{3} ions were derived from inspection of the 0 Har er section and con rmed by e amination of the 0 and 0 Har er sections. Although native Ta P is e pected to be coordinated to four metal ions, analysis of the Patterson map indicated that only three had been replaced by Ho^{3} with detectable occupancy. The presence of the e pected set of cross vectors for the three sites was con rmed using a program written for this purpose. Table 3 presents the vector pattern obtained as a percentage of the e pected pattern. nantio morph de nition was achieved by comparison with the e pected metal distribution at the active sites of the pol and nuc domains. Attempts to use the positions of Ho^{3} ions in phasing were not successful, most li ely owing to the poor resolution of the derivative data. Their use was thus limited to con rming the position of the nuc domain obtained through molecular replacement; in this instance this was of signi cant value.

The program (Jones *et al.*, 1991) was employed for all model building into $2 - _{c}$ 2 $- _{c}$ and 3 $- 2 _{c}$ electron density maps calculated using σ_A weighted coef cients (Read, 19) whenever appropriate. was used for all re nement calculations, with cross validation being performed with free values (Brunger, 1992) calculated for of the re ections chosen at random between and 2. A. nitial rigid body re nement of the MR solution (value 0. 01) was followed by re nement of the pol and v edit (residues 302– 32) and nuc (residues 12–2 0) domains as separate rigid segments using diffraction data between and 3. A. The connecting region between the two domains, appro imately residues 2 1–301, was omitted as its position was e pected to be signi cantly different from that in reported structures, because of the different relative orientation of the nuc domain. Signi cant decreases in the value (0.31) and the free value (0.37) were evident at the end of this stage. Restrained positional re nement with gradual e tension of diffraction data to 2. A was then carried out using standard (Brunger *et al.*, 1990) slow cooling protocols and the ngh and Huber parameter set (ngh & Huber, 1991). The magnitude of a was initially set by the chec procedure and several rounds of model building were also performed between re nement cycles. Difference maps calculated at this stage (0.2 , $_{free}$ 0.327) showed clear density for the connecting region and a model was easily built into this density. Subse uent positional re nement followed by tightly restrained factor re nement resulted in a nal value of 0.192 with a free value of 0.2 . (Jones *et al.*, 1991) was also used to produce ig. 1 and *A* (icholls *et al.*, 1991) was used to produce all other gures.

. Results and discussion

. . Model uality

ig. 1 shows part of a $2 - _{c}$ map in the region of the connector residues (2 1–302) between the pol and nuc domains. The current model includes coordinates for residues 10– and 70– 32 of the en yme; density for residues

– 9, part of heli , was not visible in the maps. valuation with C C (Las ows i *et al.*, 1993) indi cates that the uality of the model compares favorably with other structures re ned at 2. A, with 0 of the residues in the most favoured regions, 1 .9 in additional allowed regions, 2.3 in generously allowed regions and 1. in disallowed regions of the Ramachandran map. Because 1 of the 32 residues of the full length en yme are not seen in the electron density map, this places a total of 13 residues in disallowed regions. The best interpretation of the density that we can ma e, after several cycles of re nement and omit map calculations, at this time places these residues in their current positions. n addition, all of them ma e at least one main chain hydrogen bonding interaction with another residue that is not itself in a disallowed region. The r.m.s. deviation of bond lengths from e pected values is 0.01 A and that of bond angles is 1. 0°. The average factor for main chain atoms is 32 A^2 and that for side chain atoms is 3 A^2. The r.m.s. deviation of factors between bonded atoms is .2 A^2. The nal and $_{free}$ values as a function of resolution are given in Table 1.

.2. Co parison ith other a poly erase structures

Two other structures of the whole en yme have been reported a native structure (Kim *et al.*, 199) and that of a D A comple (om *et al.*, 199). n addition, the structure of a comple of the en yme with an inhibitory antibody has also been reported by us (Murali *et al.*, 199). n both the native and D A bound structures the relative orientations between the pol and nuc domains are similar, with the two active sites separated by ~70 A. ig. 2 shows the difference in the orientation of the nuc domain with respect to the pol domain between the current structure and those reported previously. The orientation of the nuc domain seen in this structure is also identical to that seen in the structure of the ab comple (Murali *et al.*, 199), strongly suggesting that the orientation is not driven by interaction of the ab with Ta P in that struc ture. There are other differences between this structure and that of the ab comple ; they are, however, largely con ned to the region of interaction between the en yme and the anti body. n particular, one of the long helices, heli , e ists in a signi cantly different conformation in the ab comple structure from all other Ta P structures (Murali *et al.*, 199). Analysis using the program (Hayward & Berendsen, 199) indicated that the displacement of the nuc domain is not a hinge bending motion. t is a rigid body screw motion of the nuc domain, with a rotational component of 1 3° and a translation component of ~ A along a direction perpendicular to the line joining the centers of mass of the two domains. There is little difference in chain folding within each of the domains from that of their counterparts in the reported native structure (Kim *et al.*, 199). The r.m.s. deviations for 2 0 C$^\alpha$ atoms in the nuc domain is 0.9 A between the two structures, that for 132 C$^\alpha$ atoms in the v edit domain is 1. A and that for 0 C$^\alpha$ atoms in the pol domain is 0. A. Structure of the core of the nuc domain also conforms closely to that reported for other ′–3′ e onuclease domains (Artymiu *et al.*, 1997). Signi cant changes in chain folding between the reported native structure and the current structure are, however, seen in the connector region (residues 27 –300) between the pol and nuc domains. The change in chain direction that results in the new orientation for the nuc domain occurs in a loop region (residues 291–29) in both structures. Analysis using (Hutchinson & Thornton, 199) indicates that residues 279–2 , which formed a 3_{10} heli in the reported structure, also change their conformation signi cantly. Although residues 279–2 1 retain their conformation, subse uent residues (2 1–2) change into two successive type β turns, resulting in a more e tended structure for this part of the chain.

nteraction between the two domains is more e tensive than that in the reported structure of the native en yme (Kim *et al.*, 199) and the D A comple (om *et al.*, 199), but is identical to those seen in the ab comple (Murali *et al.*, 199). This is evidenced by the larger buried solvent accessible surface area (Connolly, 19 3) calculated using a 1. A probe in *ACC* (Hubbard & Thornton, 1993) of 3 A^2, compared with 0 A^2 for the native structure. The interaction also appears to be highly complementary, judged by the nearly e ual amounts of surface area buried by the pol (173 A^2) and the nuc (191 A^2) domains and as shown in ig. . An e tensive set of van der aals interactions and hydrogen bonds stabili es the interaction. The interaction is dominated by hydrophilic contacts of the 22 residues involved in the nuc domain, 13 are hydrophilic (Arg, Asp Asn, lu ln, His and Lys); similarly, 1 of 2 residues involved in the pol domain are hydrophilic. Helices (see Kim *et al.*, 199 , for secondary structure designation) *A*′, ′, ′, ′ and ′ as well as the loop between heli ′ and strand ′ from the nuc domain interact with helices , a and as well as the loop between strand 11 and heli in the pol domain.

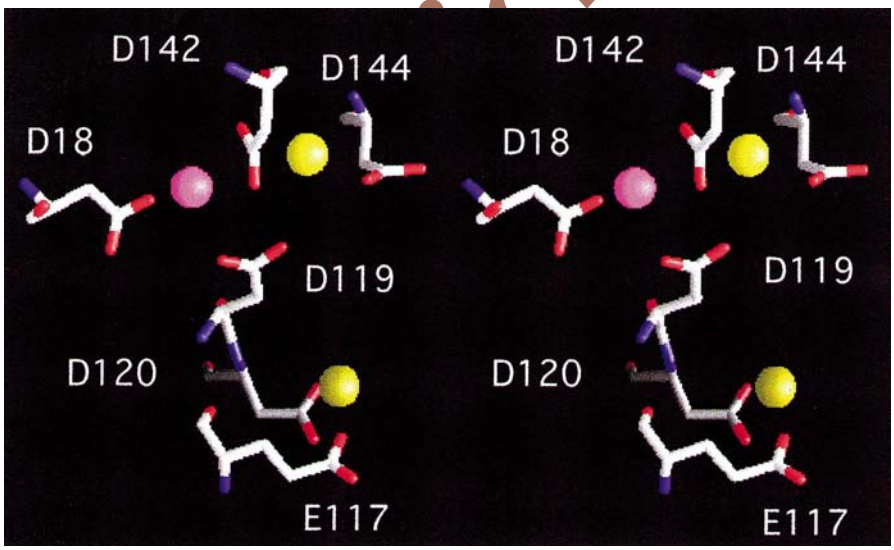
. . Metal ion distribution

n displaying the Ho^3 coordinates along with the re ned coordinates of the en yme, it was obvious that two of the three metal ions in the nuc domain and the one ion e pected to be bound at the pol site in the absence of a nucleoside triphos phate substrate had been replaced by Ho^3 . ig. 3 shows the positions of the two Ho^3 ions in the nuc domain with a Mn^2 from an earlier structure (Kim *et al.*, 199) superimposed. Appro imately ten aspartate and glutamate residues are strongly conserved among the nuc domains of D A poly merases and are presumed to be important as ligands for catalytic metal ions. t has also been shown in the reported structure of native Ta P that si of these, Asp1 , lu117, Asp119, Asp120, Asp1 2 and Asp1 , indeed provide ligands to a total of three metal ions at the nuc active site. Asp1 , Asp119 and Asp1 2 are part of the set of ligands of a n^2 ion at site , Asp1 2 and Asp1 ligand a Mn^2 ion at site and lu117, Asp119 and Asp120 provide ligands for a second Mn^2 ion at site . ne of the two Ho^3 sites (site *A*) appears to be within liganding distance (2. A) of Asp1 (D2) and Asp1 2 (D1 and D2) in the present structure; it thus seems

to correspond to the Mn^{2} ion labeled site in the earlier structure (Kim *et al.*, 199). Similarly the second Ho^{3} ion (site) in the nuc domain is within liganding distance of lu117 (1) and Asp120 (D1 and D2). Although it is not within liganding distance of Asp119 as the Mn^{2} at site of the reported structure is, there is little doubt that it corresponds to that Mn^{2} ion. This identi cation follows from the observation that the two Mn^{2} ions are ~10 A apart in the reported structure, while the n^{2} is ~ and 10 A, respectively, from Mn^{2} ions at sites and . n this structure, the two Ho^{3} ions at sites *A* and are 11. A apart. Since sites and are only A apart and the data used for the Patterson analysis did not e tend beyond A, the analysis was repeated after placing the Ho^{3} at site *A* at the coordinates of the n^{2} at site . However, only 1 of the e pected vector set was placed in positive density, supporting the above interpretation. Substi tution of only two of three metal ions at the nuc active site by Ho^{3} might perhaps be attributable to the greater charge on Ho^{3} , which, in the absence of additional shielding, is li ely to prevent simultaneous occupation of sites that are no more that A apart. The position of the third Ho^{3} ion is at the active site of the pol domain. n the native structure, the pol domain is e pected to bind one Mg^{2} ion at its active site in the absence of the nucleoside triphosphate substrate. denti ca tion of the ligands (Asp7 D1 and D2 and lu7 D1) of the third Ho^{3} suggests that it replaces this Mg^{2} ion at the pol active site.

. . Biological signi cance

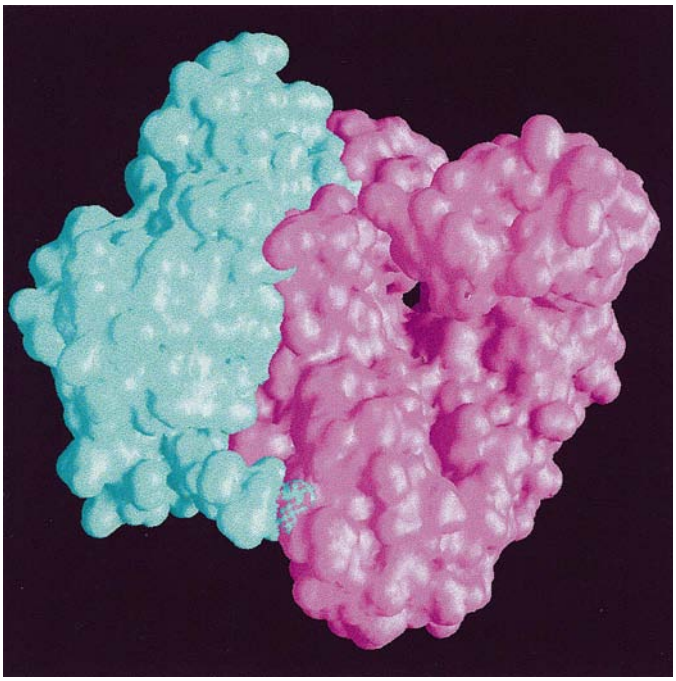
The structural basis of template directed polymeri ation of nucleotides has been e tensively documented in several polymerases (Joyce & Steit , 199 ; Marians, 1992). A number of small and large scale movements of side chains and main chain are critical for the productive binding of substrates and the catalytic cycle (Pelletier *et al.*, 199 ; Joyce & Steit , 199 ; Sawaya *et al.*, 1997; Li, Kong *et al.*, 199 ; Li, Korolev *et al.*, 199 ; Brautigam & Steit , 199 ; Doublie *et al.*, 199 ; Kiefer *et al.*, 199). However, the structural basis of primer e cision and nic translation, cataly ed predominantly by the structure speci c nuclease domain, is less well understood (Lyamichev *et al.*, 1993). Biochemical e periments have strongly suggested that this nuclease domain recogni es and binds to speci c structural elements near the polymeri ation site(s) in D A (Lyamichev *et al.*, 1993). t has also been suggested from those e periments that productively aligned pol and nuc sites are li ely to be separated by no more than – nucleotides; *.e.* ~30– 0 A, assuming an e tended D A chain (McPherson *et al.*, 19 0). However, biochemical e periments do not de ne the position and orientation of the nuc domain with respect to the pol domain. t is clear that the relative orientation in the native (Kim *et al.*, 199) and the D A comple (om *et al.*, 199) structures of Ta P places the two active sites consider ably farther apart (70 A) than e pected for productive align ment. t is not clear, however, in the absence of other data, whether the closer pro imity of the two active sites seen in the present structure and in the ab comple (Murali *et al.*, 199) is more physiologically meaningful. Both conformations of the nuc domain might be mandated by crystal pac ing forces in their respective unit cells. However, in the structure of Ta P comple ed with an ab (Murali *et al.*, 199), the relative orientation of the nuc domain is identical to that seen in the present structure, in a completely different crystal pac ing environment. denti cation of functionally productive relative orientations of the pol and nuc domains must await determination of the structure of the en yme with an appropriate D A ligand at the nuc active site. evertheless, the large difference seen in the relative orientation of the nuc domain docu mented here suggests the e istence of e traordinary e ibility between the two domains. Thus, it is clearly possible for the two active sites to approach as closely as re uired by current models for primer e cision and nic translation (Lyamichev *et al.*, 1993).



igure
A stereo picture of metal ions and their ligands in the nuc domain. The two Mn^{2} ions (yellow) which were replaced by Ho^{3} for optimi ed anomalous diffraction e periments and the n^{2} ion (magenta) are shown. The n^{2} was not replaced by Ho^{3} and is not seen in the present structure. ts position is estimated from that in the earlier structures using a rigid body transformation which relates the old and new positions of C^{α} atoms in the nuc domain. Aspartate and glutamate residues that are ligands to the metal ions are also shown (C atoms are in white, atoms red and atoms blue).

. Conclusions

Although the structures of several D A polymerases and their comple es have been determined, the structural basis of primer e cision and nic translation by the nuc domain remains obscure. e present here the structure of native Ta P determined using molecular replacement and validated by anomalous diffraction measurements made at the holmium



igure
Surface complementarity between the pol and the nuc domain in its new orientation. A solvent accessible surface using a 1. A probe is shown for the pol (magenta) and nuc (cyan) domains.

edge. Although the orientation of the nuc domain relative to that of the pol domain is dramatically different in our struc ture, it nevertheless does not provide a structural basis for interpretation of functional observations. Both orientations observed in crystal structures might be dictated by crystal pac ing forces rather than by functional considerations. denti cation of a functionally meaningful mutual orientation of the two domains must await the determination of the structure of the en yme with a suitably structured D A ligand.

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References

Arnheim, . & rlich, H. (1992). *A . e . c e .* , 131–1 .
Artymiu , P. J., Ces a, T. A., Suc , D. & Sayers, J. R. (1997). *cle c Ac s es.* **5**, 22 – 229.
Beese, L. S. & Steit , T. A. (1991). . , 2 –33.
Brautigam, C. A. & Steit , T. A. (199). *C rr. . tr ct. l.* , – 3.
Brunger, A. T. (1992). *at re* , **55**, 72– 7 .
Brunger, A. T., Kru ows i, A. & ric son, J. . (1990). *Acta Cryst.* A , – 93.
Collaborative Computational Project, umber (1993). *Acta Cryst.* D**5** , 7 0–7 3.
Chien, A., dgar, D. B. & Trela, J. M. (197). . *acter l.* , 1 0–1 7.
Connolly, M. L. (19 3). *c e ce*, , 707–713.
Decorte, R. & Cassiman, J. J. (1993). . *e . e et.* , 2 – 33.
Doublie, S., Tabor, S., Long, A. M., Richardson, C. C. & llenberger, T. (199). *at re* , , 2 1–2 .
ngh, R. A. & Huber, R. (1991). *Acta Cryst.* A , 392– 00.
om, S. H., ang, J. & Steit , T. A. (199). *at re* , , 27 –2 1.
Hayward, S. & Berendsen, H. J. C. (199). *r te s*, , 1 –1 7.
Hendric son, . A. (1991). *c e ce*, **5** , 1– .
Huang, H., Chopra, R., erdine, . L. & Harrison, S. C. (199). *c e ce*, , 1 9–1 7 .
Hubbard, S. J. & Thornton, J. M. (1993). *ACC* program. Department of Biochemistry and Molecular Biology, University College, London.
Hutchinson, . . & Thornton, J. M. (199). *r te c* . **5**, 212–220.
Jones, T. A., ou, J. ., Cowans, S. . & Kjeldgaard, M. (1991). *Acta Cryst.* A , 110–119.
Joyce, C. M. & Steit , T. A. (199). . *acter l.* , 321– 329.
Kaledin, A. S., Slyusaren o, A. . & orodets ii, S. . (1979). *c e stry sc* , , 7– 2.
Kiefer, J. R., Mao, C. & Beese, L. S. (199). *at re* , , 30 –307.
Kim, ., om, S. H., ang, J., Lee, D. S., Suh, S. . & Steit , T. A. (199). *at re* , , 12– 1 .
Korolev, S., ayal, M., Barnes, . M., Di Cera, . & a sman, . (199). *r c. atl Aca . c . A*, , 92 –92 .
Krishna Murthy, H. M. (199). *et s lec lar l y*, ol. , edited by C. Jones, B. Mulloy & M. R. Sanderson, pp. 127–1 2. Totowa, ew Jersey, USA Humana Press.
Krishna Murthy, H. M., Hendric son, . A., rme Johnson, . H., Merritt, . A. & Phi ac erley, R. P. (19). . *l. C e .* , 1 30–1 3 .
Las ows i, R. A., MacArthur, M. ., Moss, D. S. & Thornton, J. M. (1993). . *A l. Cryst.* , 2 3–291.
Lawyer, . C., Stoffel, S., Sai i, R. K., Myambo, K., Drummond, R. & elfand, D. H. (19 9). . *l. C e .* , 27– 37.
Li, ., Kong, ., Korolev, S. & a sman, . (199). *r te c* . , 111 –1123.
Li, ., Korolev, S. & a sman, . (199). . , 7 1 –7 2 .
Lyamichev, ., Brow, M. A. & Dahlberg, J. . (1993). *c e ce*, , 77 –7 3.
McPherson, A., Jurna , ., ang, A., Kolpa , . & Rich, A. (19 0). *ys.* . , 1 –173.
Marians, K. J. (1992). *A . e . c e .* , 73–719.
Murali, R., Shar ey, D. J., Daiss, J. L. & Krishna Murthy, H. M. (199). *r c. atl Aca . c . A*, **5**, 12 2–12 7.
ava a, J. (199). *Acta Cryst.* A**5** , 1 7–1 3.
icholls, A., Sharp, K. A. & Honig, B. (1991). *r te s*, , 2 1–29 .
twinows i, . & Minor, . (1997). *et s y l.* , 307–32 .
Pelletier, H., Sawaya, M. R., Kumar, A., ilson, S. H. & Kraut, J. (199). *c e ce*, , 1 91–1903.
Read, R. J. (19). *Acta Cryst.* A , 1 0–1 9.
Sawaya, M. R., Prasad, R., ilson, S. H., Kraut, J. & Pelletier, H. (1997). *c e stry*, , 1120 –1121 .
Tic le, . J. (1992). *r cee s t e CC t y ee e . lec lar e lace e t*, edited by . J. Dodson, S. lover & . olf, pp. 20–32. arrington Daresbury Laboratory.
Urs, U. K., Shar ey, D. J., Peat, T. S., Hendric son, . A. & Krishna Murthy, H. M. (199). *r te s*, , 111–11 .
right, P. A. & ynford Thomas, D. (1990). . *at l.* , 99–117.