- & Prakash, L. *Nature* **406**, 1015–1019 (2000).
2. Tissier, A., McDonald, J. P., Frank, E. P. & Woodgate, R. *Genes Dev.* **14**, 1642–1650 (2000).
 3. Nair, D. T., Johnson, R. E., Prakash, S., Prakash, L. & Aggarwal, A. K. *Nature* **430**, 377–380 (2004).
 4. Dreyer, G. B. et al. *Biochemistry* **31**, 6646–6659

- (1992).
5. Murthy, K. H., Winborne, E. L., Minnich, M. D., Culp, J. S. & Debouck, C. J. *Biol. Chem.* **267**, 22770–22778 (1992).
 6. Eom, S. H., Wang, J. & Steitz, T. A. *Nature* **382**, 278–281 (1996).

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DNA POLYMERASES

Nair et al. reply

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We have solved the crystal structure of human DNA polymerase- ϵ (hPol ϵ) bound to a template primer and incoming nucleotide, and shown that hPol ϵ uses Hoogsteen base-pairing for normal DNA synthesis¹. Jimin Wang questions the space group we used and claims that Hoogsteen base-pairing is not supported by our electron-density maps². We show here that the issue of space group does not affect our conclusions¹ and that our electron-density maps are clear in indicating that Hoogsteen and not Watson–Crick base-pairing occurs in this situation.

We are aware that our data merge well with 6/*mmm* symmetry and, in fact, we calculated the initial experimental (MAD-phased) map in space group *P*₆₅22. The map revealed two DNA polymerases, one at the replicative end of the template primer and the other at the blunt end. This was unexpected as there was no precedence for such a binding arrangement. Because the two ends are chemically and structurally distinct (for example, the replicative end has unpaired template bases and a bound incoming nucleotide, whereas the blunt end does not), the two polymerases could bind slightly differently, and they do. We therefore refined the structure in space group *P*₆₅ rather than *P*₆₅22, so as not to force an exact identity on the two polymerases.

In addition, data from a brominated derivative indicated that the complex packs in two orientations — ‘up’ in about half the unit cells and ‘down’ in the others, as occasionally occurs in crystals of protein–DNA complexes³. We therefore refined the structure in *P*₆₅ with two orientations — as the more prudent approach to the packing in crystals. However, whether the structure is refined in space group *P*₆₅ or *P*₆₅22 has no bearing on our conclusion that replication by hPol ϵ occurs by Hoogsteen and not Watson–Crick base-pairing¹.

This conclusion is based on electron-density

maps, which also include simulated annealing omit maps — the gold standard for the evaluation of structure. For the calculation of such maps, the structure (with the template nucleotide omitted) is heated to 1,000 K and then slowly cooled to remove model bias. Figure 1 shows a portion of the simulated annealing 2*F*_o–*F*_c omit map contoured at 0.8 σ . The only way to fit this and other maps is with a Hoogsteen base pair: for a Watson–Crick base pair, either the sugar phosphate is far out of density or the bases are too close, with non-linear and short hydrogen bonds and a C1′–C1′ distance that is much smaller than the expected distance of about 10.5 Å.

It is not clear how Wang calculates his maps², but almost any base-pair arrangement could be fitted at these low σ values (compare his Fig. 1c,d at 0.5 σ and 0.2 σ , respectively, with ours contoured at 0.8 σ). Even then, the Watson–Crick base pair seems to us to be rather ill-fitted, with short and nonlinear hydrogen bonds. A single base pair makes a minor contribution to the overall scattering, and therefore can be refined in any arrangement without any significant effect on *R*-factor or *R*-free. The geometry of our structure is reasonable¹ (r.m.s.d._{bond} = 0.006 Å; r.m.s.d._{angle} = 1.3 deg) and there are no steric clashes flagged during refinement. Wang points to a distance of 2.4 Å to the O5′ atom (his Fig. 1c), but this is a ‘terminal’ atom added by the refinement program and so can be moved slightly.

Wang claims² that, as G–C cannot form a Hoogsteen base pair at physiological pH, Hoogsteen base-pairing is unlikely to be used in replication by hPol ϵ . However, we find that in the structure of Pol ϵ bound to template guanine and an incoming dCTP, Hoogsteen base-pairing does occur¹. Also, in experiments using N7-modified analogues of purine template bases, where modifications at position N7 disrupt Hoogsteen but not Watson–Crick

Figure 1 | Hoogsteen base-pairing (dA•dTTP) in the active site of human DNA polymerase- ϵ .

A simulated annealing 2*F*_o–*F*_c omit map, contoured at 0.8 σ , is shown. The templating nucleotide was omitted and the structure heated to 1,000 K and then cooled (40 K per step). **a, b**, Whereas a Hoogsteen base pair (**a**) fits the density, a Watson–Crick base pair (**b**) does not. In **b**, the bases are too close (top) or the sugar phosphate is far out of density (bottom).

base-pairing, we find strong inhibition of DNA synthesis by Pol ϵ , but not by all other DNA polymerases of the A, B or Y families⁵.

Our structural and biochemical evidence shows that Pol ϵ is exceptional among the known DNA polymerases in its dependence on Hoogsteen base-pairing for DNA synthesis.

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1. Nair, D. T., Johnson, R. E., Prakash, S., Prakash, L. & Aggarwal, A. K. *Nature* **430**, 377–380 (2004).
2. Wang, J. *Nature* **437**, doi:10.1038/nature04199 (2005).
3. Becker, S., Groner, B. & Muller, C. W. *Nature* **394**, 145–151 (1998).
4. Nair, D. T., Johnson, R. E., Prakash, L., Prakash, S. & Aggarwal, A. K. *Structure* (in the press).
5. Johnson, R. E., Prakash, L. & Prakash, S. *Proc. Natl Acad. Sci. USA* **102**, 10466–10471 (2005).

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