a C. hPolι incorporates a C opposite this lesion as efficiently as opposite an undamaged G, thereby providing an effective means for replication through such minor-groove adducts[24]. □

Methods

Protein and DNA preparation

The glutathione *S*-transferase (GST) and hPolι (residues 1–420) fusion protein was expressed in yeast strain BJ5464 from plasmid pBJ941, and then purified and cleaved as described for yeast Polη[13]. To prepare selenomethionine (SeMet)-labelled hPolι, we expressed plasmid pBJ1066 in *Escherichia coli* strain B834, which is auxotrophic for methionine, and grew the cells in M9 minimal medium. The primer was synthesized as 12- and 13-nucleotide oligonucleotides, with the latter containing a dideoxycytosine at its 3′ end (5′-GGGGGAAGGACCC^{dd}-3′). These were annealed with an 18-nucleotide template (5′-TTCTAGGGTCCTTCCCCC-3′) to yield 12/18 and 13/18 primer templates. The template was also synthesized with three thymines substituted by 5-bromouracil (5′-TTCAAGGGU^{Br}CCU^{Br}U^{Br}CCCCC-3′), and then annealed with the 13-nucleotide primer to yield a 13/18(Br) primer template.

Cocrystallization

We obtained three different cocrystals: SeMet, Native1 and Native2. SeMet cocrystals were obtained by incubating SeMet hPolι with the 12/18 primer template in a molar ratio of 1:1.2, plus 5 mM ddTTP and 5 mM $MgCl_2$. Native1 cocrystals were obtained by mixing native hPolι with the 13/18 primer template plus 5 mM ddTTP and 5 mM $MgCl_2$. The Native2 cocrystals were obtained by incubating native hPolι with the 13/18(Br) primer template plus 15 mM dTTP and 5 mM $MgCl_2$. For each, the complex was crystallized from solutions containing 10–15% PEG 5000 MME and 0.2–0.4 M $(NH_4)_2SO_4$ in 0.1 M MES buffer (pH 6.5). The three cocrystals belong to the space group $P6_5$, with identical cell dimensions of $a = 98.5$ Å, $b = 98.5$ Å, $c = 203.7$ Å and $\alpha = \beta = 90°$, $\gamma = 120°$.

Structure determination

Multiwavelength anomalous diffraction (MAD) data on cryo-cooled SeMet cocrystals (2.6 Å) were measured at the Advanced Photon Source (APS, beamline 14-ID) at three wavelengths, corresponding to the edge and peak of the Se *K* edge absorption profile plus a remote point (Supplementary Table 1). The Native1 and Native2 data were also measured at the APS at beamlines 14-ID (2.1 Å) and 17-ID (2.3 Å), respectively. We found the positions of nine selenium atoms from the SeMet data using the program SOLVE[25]. The initial experimental phases (2.8 Å) from these selenium positions were applied to Native1 data, and then extended to 2.1 Å with solvent flattening by the program CNS[26]. This yielded a readily interpretable electron density map that was used to build the initial complex. Unexpectedly, two hPolι molecules were bound to the DNA, related by a pseudo-dyad axis perpendicular to the DNA axis. The two hPolι molecules were built independently and refined, but there was no density for the incoming nucleotide.

The Native1 structure was used as a molecular replacement model to phase the Native2 complex (crystallized with brominated DNA and dTTP instead of ddTTP) with the program AmoRe[27]. The Native2 structure showed clear electron density for the incoming dTTP. Anomalous difference Fourier maps revealed six bromine peaks, suggesting that the complex packed in two orientations in the crystal. The Native2 structure was refined in two orientations with CNS, with the bromine atoms helping to fix the register of the DNA. After iterative rounds of refinement, model building with program O[28] and water picking, the R_{free} dropped to 28.6%. The final Native2 model includes residues 27–414 for molecules A and B, DNA (electron density for four of the five unpaired template residues towards the 5′ end was not visible), dTTP and 438 water molecules. The model has good stereochemistry: 82% of the residues are in the most favoured regions of a Ramachandran plot and only 0.5% are in the disallowed regions.

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Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to A.K.A (aggarwal@inka.mssm.edu). Coordinates have been deposited in the Protein Data Bank under accession code 1T3N.

erratum

Evidence for dynamically organized modularity in the yeast protein–protein interaction network

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