Genomic integrity is threatened by cytotoxic DNA double-strand breaks (DSBs), which must be resolved efficiently to prevent sequence loss, chromosomal rearrangements/translocations, or cell death. Polymerase μ (Polμ) participates in DSB repair via the nonhomologous end-joining (NHEJ) pathway, by filling small sequence gaps in broken ends to create substrates ultimately ligatable by DNA Ligase IV. Here we present structures of human Polμ engaging a DSB substrate. Synapsis is mediated solely by Polμ, facilitated by single-nucleotide homology at the break site, wherein both ends of the discontinuous template strand are stabilized by a hydrogen bonding network. The active site in the quaternary Polμ complex is poised for catalysis and nucleotide incorporation proceeds in crystallo. These structures demonstrate that Polμ may address complementary DSB substrates during NHEJ in a manner indistinguishable from single-strand breaks.
Genomic DNA is vulnerable to damage/breakage caused by exogenous exposures to ionizing radiation or endogenous reactive oxygen species generated through cellular metabolism. When phosphodiester backbone breaks on opposing DNA strands cluster, cytotoxic DNA double-strand breaks (DSBs) form. Persistently unrepair DSBs in DNA can have disastrous consequences, leading to human cancers and other diseases. Alternatively, DSBs can be systematically generated in a programmed manner, as observed in the V(D)J recombination pathway required for immunoglobulin gene maturation. DSBs are repaired by multiple pathways, including nonhomologous end-joining (NHEJ), which is favored in nonreplicating cells, or in cells lacking replicated sister chromatids. The multiprotein NHEJ complex binds and bridges broken ends so they can be ultimately rejoined. Its assembly is presumed to occur in a stepwise fashion, wherein the Ku70/80 heterodimer first identifies and binds the ends, in concert with the DNA-PK catalytic subunit. This core complex, known as the DNA-PK holoenzyme, enlists other protein binding factors—DNA Ligase IV, XRCC4, Artemis, XLF, and various polymerases—to subsequently process and rejoin the ends, depending on their sequence and structural composition.

The Family X polymerases (Pols), Polλ, Polμ, and terminal deoxynucleotidyl transferase (TdT), are recruited to the NHEJ complex by means of an N-terminal BRCT protein–protein interaction domain. All three Family X enzymes are involved in V(D)J recombination, though with distinct expression and polymerization profiles. Polλ and μ are widely expressed, primarily template-dependent polymerases, which function in maturation of immunoglobulin heavy- and light-chain loci, respectively. In contrast, TdT expression is strictly limited to immunological cells, where it functions as a primarily template-independent polymerase contributing to gene sequence diversity during V(D)J recombination. In addition to their specific roles in V(D)J recombination, Pols λ and μ also function more broadly in classical NHEJ. While both polymerases can utilize complementary DSB substrates with paired primer termini, Polμ is uniquely capable of bridging broken DNA ends lacking break site microhomology. Though there exists a plethora of biochemical and structural information illustrating the activity of these Family X polymerases on single-strand break (SSB) substrates, understanding how each individual polymerase copes with DSB end-bridging is unclear. Thus far, only DSB-bound crystal structures of murine TdT and a chimeric construct of murine TdT containing the Loop1 region from murine Polμ are currently available, providing an important yet incomplete portrait of Family X polymerase behavior during NHEJ. We therefore present high-resolution crystal structures of the human Polμ catalytic domain simultaneously engaging both ends of a complementary DSB substrate. A catalytically-poised pre-catalytic quaternary complex was trapped using a correctly paired nonhydrolyzable nucleotide, which could be exchanged with a hydrolyzable nucleotide to generate “snapshots” of the incorporation reaction proceeding in crystallo. These structures indicate that Polμ provides a rigid scaffold, which can

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**Fig. 1 Structural characterization of hPolμΔ2 engaging a complementary DSB substrate.**

- **a** DNA substrate crystallized with hPolμΔ2 and nonhydrolyzable dUMPNNP. Template strand discontinuity is marked (dashed red line).
- **b** Pre-catalytic quaternary DSB (protein, dark green; DNA and dUMPNNP colored as in a; template break site, red) The ordered ends of Loop1 are indicated (Gln364/Ala384).
- **c** hPolμΔ2 pre-catalytic quaternary complex active site. 2Fo-2Fc electron density (gray mesh) contoured at 1σ. Ionic interactions (Mg2+ ion, purple; water molecules, red) are indicated by black bars. Interatomic distance between 3'-OH and α-phosphate (dashed cyan line) was measured in PyMOL (Schrödinger).
- **d** Zoomed-in view of the hPolμΔ2 active center, with arrows indicating putative electron movements during reaction progression.
accommodate both SSBs and complementary DSBs in a nearly indistinguishable manner.

Results

Pre-catalytic hPolαΔ2 quaternary complex with complementary DSB. A variant of the Pol μ catalytic domain with increased crystallizability and biochemical characteristics equivalent to those of the wildtype enzyme was used for this study (henceforth, referred to as hPolαΔ221). In this variant, the flexible, non-conserved loop (Loop2) between β-strands 4 and 5 of the palm subdomain was deleted (ΔPro398–Pro410), and the ends of the β-strands were fused by a single glycine residue (Gly410). The hPolαΔ2 pre-catalytic quaternary complex was incrementally assembled by first incubating the protein with an annealed downstream DNA duplex containing a 5′-phosphorylated downstream primer (Fig. 1a) to dictate correct binding, as Pol μ prefers to orient the nascent base pair binding site using the 5′-end of the gap rather than the 3′-end20, and its activity is stimulated by the presence of a phosphate at that position15. Synapsis is then achieved by the polymerase after addition of annealed upstream DNA—facilitated by 3′-primer terminal single-nucleotide complementarity (A:T), consistent with reports that Polμ efficiently mediates end-bridging without other NHEJ-binding partners25. Binding a correctly paired nonhydrolyzable nucleotide analog 2′-deoxyuridine-5′-[(α,β)-imido]triphosphate (dUMPNNP) in the active site leaves the enzyme poised for catalysis (PDB ID code 6WIC, Fig. 1b and Table 1). The upstream and downstream duplex regions are positioned distally from one another, an arrangement induced by an ~90° bend in the template backbone downstream duplex rather than the 3′-end20, and its activity is sti-

| Table 1 Data collection and refinement statistics. |
|-----------------------------------------------|
| **Pre-catalytic** | **Incomplete incorporation** | **Post-catalytic** |
| complexa,b     | b                  | complexa,b       |
| PDB ID code    | 6WIC               | 6WID             | 6WIE             |
| **Data collection** |                    |                  |                  |
| Space group    | P2,2,2,1           | P2,2,2,1         | P2,2,2,1         |
| **Cell dimensions** |                  |                  |                  |
| a, b, c (Å)    | 60.13, 60.19, 60.07 | 62.23, 118.05, 62.04, 118.32 | 62.25, 118.32 |
| A, β, γ (°)    | 60.13, 60.19, 60.07 | 62.23, 118.05, 62.04, 118.32 | 62.25, 118.32 |
| **Resolution (Å)** | (1.58–1.55)c       | (1.53–1.50)       | (1.53–1.50)       |
| Rsym (%)       | 5.2 (5.35)         | 5.3 (5.48)       | 10.6 (5.75)       |
| /fo–fc (%)     | 36.63 (1.5)        | 34.28 (2.24)     | 18.54 (1.77)      |
| Completeness (%) | 98.5 (98.1)       | 98.9 (99.9)      | 99.8 (99.8)       |
| Redundancy     | 6.4 (4.5)          | 6.0 (5.5)        | 6.3 (5.4)         |
| **Refinement** |                    |                  |                  |
| Resolution (Å) | 3.15–1.55          | 3.10–1.50        | 3.05–1.50         |
| No. reflections | 64,729             | 71,176           | 71,906            |
| Rwork/Rfree (%) | 16.55/17.94       | 16.57/16.95      | 16.99/16.88       |
| Incorporation extent | 0%                  | 40%              | 100%              |
| No. of atoms | Protein 2563        | 2562             | 2564              |
| DNA 379        | 420d              | 399              |
| Nucleotide 28e | 28e/9f            | 9f              |
| Water 347      | 348               | 346              |
| B-factors | Protein 22.89       | 18.38            | 20.17             |
| DNA 19.06      | 13.87f            | 15.45            |
| Nucleotide 14.20f | 9.21f/14.56f     | 25.08f           |
| Water 33.38    | 31.09             | 31.04            |
| R.m.s. deviations | 0.009            | 0.009            | 0.009             |
| Bond angles (°) | 1.038             | 1.087            | 1.030             |

*a* A single crystal was used to collect each data set.

*b* These crystals were collected on the Southeast Regional Collaborative Access Team (SER- CAT) 22-ID beamline at the Advanced Photon Source at Argonne National Laboratory.

*c* Values in parentheses are for highest-resolution shell.

Table 2). The aromatic sidechains of Trp434 and Phe389 likely stabilize by van der Waals interactions with the backbone of Lys450 (Table 2). The position of the downstream duplex is stabilized by van der Waals interactions between its nonbridging oxygens and the Thr250 sidechain, and that of the incoming dUMPNNP are observed with C3′-endo sugar puckers, which leaves the 3′-OH ideally positioned for in-line attack on the α-phosphate (3.6 Å) of dUMPNNP (Fig. 1c, d). Both divalent metal sites are occupied by Mg2+ ions, which are observed with octahedral coordination mediated by the 3′-OH, Asp330, Asp332, Asp418, the triphosphate oxygens, and associated water molecules.

Protein–DNA interactions in the hPolαΔ2 quaternary complex. From hPolαΔ2's perspective, the process of binding the downstream duplex to the 8 kDa subdomain likely occurs in a similar fashion, regardless of whether the DNA substrate contains an intact or discontinuous template strand, as relevant breaks occur upstream of the nascent base pair binding site. The ~90° bend in the template backbone between residues T4 and T5 opens the helix to allow access of incoming nucleotides to the nascent base pair binding site, positioning the 5′-phosphate on the downstream primer >20 Å from that location (Fig. 2a). This bend is reinforced by multiple putative hydrogen bonding interactions from the nonbridging phosphate oxygens of residues T4–T6 to the sidechains of Arg442, Arg449 (partially disordered), and Lys450 (Table 2). The position of the downstream duplex is stabilized by van der Waals interactions with the backbone of Gly174 on the N-terminal end of α-helix B, anchoring the 5′-phosphate to the 8 kDa subdomain via putative hydrogen bonds with the sidechains of Arg175 (partially disordered) and His208 (Fig. 2a and Table 2). Interestingly, mutations of Gly174 and Arg175, which diminish the fidelity and efficiency of Polα’s activity in NHEJ26, have been discovered in skin and ovarian cancers, respectively27,28.

Electron density for the upstream template strand clearly highlights the backbone discontinuity between residues T6 and U1 (Fig. 1c). Both sides of the break in the pre-catalytic complex are stabilized by a hydrogen bonding network within Polα’s substrate binding cleft (Fig. 2b and Table 2). The O5′ atom on upstream template residue U1 is solvent-exposed, but the O3′ atom on the 3′-end of downstream template residue T6 orients toward the protein surface and putatively hydrogen bonds with Asn457. Arg445 also lies in the minor groove within hydrogen bonding distance of the T6 base. Arg387 interacts with the U1 base upstream of the break. The U2–U3 phosphate lies within hydrogen bonding distance of the Glu386 and Arg387 backbone amide nitrogens, whereas Gln364 putatively interacts with the 3′-OH of residue U3.

Upstream primer strand positioning is stabilized by van der Waals, ionic, and hydrogen bonding interactions (Fig. 2c and Table 2). The aromatic sidechains of Trp434 and Phe389 likely stabilize the sugar moieties of the primer terminal and penultimate nucleotides, respectively, via π-CH interactions. The upstream P1–P2 phosphate is secured by multiple interactions between its nonbridging oxygens and the Thr250 sidechain, its backbone amide, and the backbone amide of Gly247. The P2–P3 phosphate is stabilized by a putative hydrogen bond with the backbone amide of Gly245 and an interaction with the Na+ coordinated by the HnH2 (helix-hairpin-helix) motif (residues Thr241–Val246) that is conserved throughout the Family X polymerases6. The P3–P4 phosphate is tethered near the catalytic...
center via a hydrogen bond with Arg416, which has been shown to be dispensable for nucleotide incorporation on SSBs but essential for DSB repair.29 The His329 sidechain, thought to bridge the primer terminal phosphate and incoming nucleotide during NHEJ19, is observed in multiple conformations, one of which lies within long-range hydrogen bonding distance of a P3–P4 phosphate oxygen. Correct positioning of the primer terminal 3′-OH is crucial for catalysis and is largely mediated by coordination with the catalytic Mg2+.

The observed hPolμΔ2 conformation is catalytically competent. Transferring the hPolμΔ2 pre-catalytic quaternary complex crystals from a cryoprotectant solution containing nonhydrolyzable dUMPNPP to a cryoprotectant solution containing hydrolyzable dTTP allows nucleotide exchange and insertion in crystallo, yielding a structure exhibiting incomplete (~40%, PDB ID code 6WIE, Table 1 and Fig. 3c) incorporation after a longer soak. Successful nucleotide incorporation within the crystalline lattice indicates that the observed pre-catalytic quaternary conformation is indeed catalytically competent. Superposition of pre- and fully post-catalytic complexes reveals no large-scale movements of protein subdomains, DNA substrate, or active site residues during nucleotide insertion (0.084 Å RMSD over 278 Cα atoms, Fig. 3d). There is a slight (1.4 Å) shift of the 3′-primer terminus toward the α-phosphate of the newly incorporated nucleotide, allowing phosphodiester bond formation. A correlated adjustment (≤1.1 Å) of the Trp434 sidechain is also observed, mediated by a rotation around the CB-CG bond (Fig. 3e). These subtle motions appear to be a normal consequence of nucleotide insertion by Polμ21 and are likely observed regardless of DNA substrate configuration. There is no observed density for a third “product metal” in these structures (Fig. 3), even in the structure exhibiting incomplete incorporation where the “product metal” might be expected, which is consistent with previously published reports.30 Upon reaction completion, the pyrophosphate leaving group becomes partially disordered and replaced by solvent molecules, which cannot be definitively modeled.

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**Table 2** Putative hydrogen bonding interactions in the hPolμΔ2 pre-catalytic quaternary complementary DSB complex.

| Region         | Interacting atoms | Interatomic distance (Å) |
|----------------|-------------------|--------------------------|
| Downstream primer strand | Arg175 NE-D1 OP1 | 3.3                      |
|                 | His208 ND1-D1 OP2 | 2.6                      |
|                 | Ser209 N-D2 OP1   | 3.0                      |
|                 | Gly206 N-D2 OP1   | 3.0                      |
|                 | His208 N-D2 OP2   | 3.0                      |
|                 | Glu207 N-D2 OP2   | 3.3                      |
|                 | His204 N-D3 OP1   | 2.9                      |
| Downstream template strand | Arg449 NE -- T6 OP1/2 | 2.6-3.3                 |
|                 | Arg442 NE–T5 OP2  | 2.9                      |
|                 | Arg442 NH2–T5 OP1 | 3.0                      |
|                 | Ly450 N2–T4 OP1   | 2.6                      |
|                 | Asn457 ND2–T6 O3’ | 2.9                      |
|                 | Arg445 NH1–T6 O2  | 2.9                      |
| Upstream template strand | Arg387 NH1–U1 N3  | 3.0                      |
|                 | Glu386 N–U3 OP1   | 2.8                      |
|                 | Arg387 N–U3 OP1   | 3.0                      |
|                 | Glu364 NE–U3 O3’  | 3.1                      |
| Upstream primer strand | Thr250 N–P2 OP1   | 3.1                      |
|                 | Thr250 OG1–P2 OP1 | 2.7                      |
|                 | Gly247 N–P2 OP1   | 2.9                      |
|                 | Gly245 N–P3 OP1   | 2.9                      |
|                 | Arg416 NH2–P4 OP1 | 2.8                      |
|                 | His329            | 3.6                      |
|                 | NE2 (A)–P4 OP1    | 3.6                      |

*Putative hydrogen bonds were assessed based on geometry and interatomic distances measured in PyMOL.*

*Note: Measured distances are approximate, since sidechains are partially disordered.*
5′-phosphate binding becomes critical on tenuous DNA substrates. Previous studies have shown that interactions with or near the 5′-phosphate on the downstream duplex can have deleterious consequences for efficiency and fidelity of end-joining activity by Polμ26,31. Disease-associated mutations of Gly174 and Arg175 demonstrated only subtle differences in polymerization efficiency on a single-nucleotide SSB substrate, which became more pronounced as the SSB became a complementary DSB. Repair of a noncomplementary DSB, which lacks microhomology at the break site to facilitate synapsis, was abrogated further26. Moreover, substitution of Arg175 with histidine showed less-deleterious effects on end-joining than did replacement with alanine31. In order to determine whether His208 (Fig.2a) binding to the 5′-phosphate is similarly required for contribution of Polμ to end-joining, we generated an alanine substitution at this position and assayed its ability to mediate single-nucleotide gap-filling on both partly complementary and noncomplementary DSB ends. Similar to previous reports of the Gly174 and Arg175 mutants, the H208A mutant demonstrated decreased end-joining efficiency on complementary DSB ends (Fig. 4, top), compared with the wildtype enzyme. Activity of this mutant was negligible when compared with a no-polymerase control when using noncomplementary overhangs (Fig.4, bottom).

Discussion

Superposition of the human hPolμΔ2 pre-catalytic quaternary complementary DSB and either the human (Fig. 5a, b and Fig. 2) or mouse (Fig. 5c, d) Polμ ternary SSB complexes reveal a high degree of global similarity (0.34 Å RMSD over 284 Ca atoms and...
0.81 Å over 287 Ca atoms, respectively). Subtle differences can be observed in the position of the Loop2 region (disordered in mouse Polμ and deleted ΔPro389-Pro410 in the human Polμ) and at the distal ends of the DNA duplex, all of which can be influenced by crystal packing and DNA pseudo-stacking between the crystal forms. Interactions between protein and either the 5′-phosphate (Fig. 2a) or upstream primer strand (Fig. 2c) are conserved between both substrate types in these structures, but slight variations are observed in residues surrounding the break in the human structures, namely the sidechain conformations of Asn457 and His459 (Fig. 2b). His459 lies within long-range hydrogen bonding distance of the T7-T8 phosphate oxygens in the SSB (3.3 Å), but likely interacts instead with Glu386 (2.7 Å) on the end of β-strand 4 near Loop1 in the DSB complex. The precise nature of these interactions and their contributions to catalysis by Polμ in SSB and DSB repair are currently unclear, as previous studies have shown that glycine substitution of His459 had no apparent effect on single-nucleotide gap-filling on either SSB or DSB substrates, and the N457D mutant was profoundly impaired on all substrates32. The similarities between the single- and DSB complexes therefore suggest that Polμ addresses DSB substrates containing break site complementarity in a manner analogous to its engagement of gapped SSB substrates, and that the polymerization reaction proceeds through the same mechanism.

Comparison of the hPolμΔ2 quaternary complementary DSB structure with those available for mouse TdT reveals distinct differences. Superposition of the hPolμΔ2 complementary DSB with that of mouse TdT bound to a DSB substrate of similar configuration (PDB ID code 5D4623) shows structural correlation for the protein (RMSD of 1.04 Å over 275 Ca atoms, Fig. 6a), but less for the DNA (Fig. 6b). Equivalent residues comprising the primer terminus/nascent base pair binding site (referred to as an A-form “mini-helix” in an otherwise B-form duplex), and the downstream duplex show very similar positioning, but duplex upstream of the primer terminal base pair (P4:T6 in hPolμΔ2) is “wedged” open in mouse TdT by insertion of Loop1 residues into the duplex. A similar phenomenon is observed in another mouse TdT DSB synaptic complex mediated by single-nucleotide complementarity on the template strand (PDB ID code 4QZ822, RMSD of 1.1 Å over 280 Ca atoms, Fig. 6c, d). The upstream primer strand in this structure is also “wedged” open, in the absence of an upstream template strand, suggesting that this behavior occurs independently of upstream base pairing in TdT (Fig. 6e). The “wedging” phenomenon is not observed in the hPolμΔ2 complementary DSB substrate.
Among the Family X polymerases, the Loop1 motif is not conserved, and, indeed, the Loop1 region from mouse Polδ (henceforth, referred to as TdT-μ) included crystal structures of the chimera in complex with a 1nt-gapped SSB substrate (PDB ID code 6GO5, Fig. 7a, b) and with a noncomplementary DSB synaptic complex reinforced by a triplex-forming oligonucleotide (PDB ID code 6GO7, Fig. 7c, d). Superposition of the hPolμΔ2 complementary DSB synaptic complex with these structures yielded some intriguing differences. As with the superpositions with TdT, comparisons of the TdT-μ chimeric complexes showed similarities in global protein structure (1.02–1.03 Å over 274–276 Ca atoms), but some differences in DNA structure. The TdT-μ crystal structure with the 1nt-gapped SSB shows strong similarity of the DNA substrate position with that of the hPolμΔ2 complementary DSB, and the “wedged open” upstream duplex is not observed in either structure (Fig. 7b). In the TdT-μ noncomplementary DSB, however, the 3′-terminal base of the downstream template strand—which could mispair with the upstream primer terminal base, but is instead disordered—leaving an unpaired primer terminus (Fig. 7d, e). As in the crystal structures of TdT, the duplex upstream of the primer terminal nucleotide is observed in a “wedged” open conformation, though not by direct intervention of the chimeric mouse Polμ Loop1 residues, as occurs in wildtype TdT. It is therefore tempting to speculate that Polμ might utilize different modes of substrate binding, based on the sequence and structure configuration at the break site. Opening of the upstream duplex in a noncomplementary DSB could provide more access of the Loop1 region to interact with and stabilize the broken template strand, but may not be required for repair of complementary DSBs.

Among the Family X polymerases, the Loop1 motif is not required for repair of complementary DSB ends, but is critical for repair of DSBs lacking microhomology12. That Loop1 is disordered in these structures is perhaps unsurprising, given that repair of DSB substrates containing as little as one complementary base pair at the break site is Loop1-independent. Loop1 is largely disordered in all available structures of human or mouse Polμ—which contrasts with structures of TdT engaging a similar complementary DSB substrate, wherein an ordered Loop1 contributes to break site interactions (Fig. 6b)23. Comparison of the Loop1 conformations in the Polμ structures, however, may provide some insight. Superposition of the hPolμΔ2 1nt-gapped SSB and complementary DSB complexes shows that, although Loop1 is disordered in both structures, its overall trajectory and extent of disorder are similar (Fig. 6b and Fig. 8, green and purple dashed lines). In contrast, however, the overall trajectory of Loop1 in the human versus the mouse Polμ SSB structures widely differ (Fig. 8, purple and blue dashed lines, respectively). Loop1 also displays different conformations in the human Polμ complexes with different SSB substrates (1nt-gapped SSB, purple and 2nt-gapped SSB, orange). Although we cannot rule out the possibility that its conformations are influenced by crystal packing interactions, Loop1 likely adopts a conformation capable of stabilizing the template strand of any given substrate configuration, which becomes essential as the extent of break site complementarity decreases.

Since the Family X polymerases must contend with both ends of their substrates, often separated by >20 Å distance owing to the ~90° bend (Fig. 2a) in the template backbone, strong interactions with the 5′-phosphate, the primer terminus, and template strands are required for correct catalytically-competent geometry. This theory is supported by a variety of mutagenesis experiments, wherein different substrate interactions have been removed and interrogated for their roles in subsequent repair. Alanine substitution mutagenesis of Arg41629 and His32919 are thought to hinder correct positioning of the primer strand for catalysis
Likewise, loss of 5′-phosphate identification and positioning owing to mutations of Gly17426, Arg17531, or His208 (Fig. 4) lead to diminished repair capacity. These mutations have increasingly profound effects as the complexity of the repair substrate increases—SSB < complementary DSB < non-complementary DSB. These results suggest that substrate binding by Polμ is multifactorial, with myriad interactions working in synergy. In more simplistic DNA substrate configurations, interactions in each key area (primer strand, 5′-phosphate, etc) serve a redundant role for the others. However, as synapsis becomes more tenuous, each individual interaction becomes critical. The DSB-bound Polμ structures presented in this study provide a greater understanding of how Polμ addresses different substrate configurations, and how interactions with these substrates contribute to DSB repair in NHEJ.

**Methods**

**Expression and purification of human Polμ constructs.** Full-length human Polμ and a crystallization variant of the human Polμ catalytic domain (hPolμΔ2; Pro132-Ala494 with Pro398–Pro410 of Loop2 deleted and replaced by Gly410) were cloned into the pGEXM vector and expressed in Rosetta2 (DE3) cells. hPolμΔ2 was expressed in Rosetta2 (DE3) cells in LB medium supplemented with 100 μg mL⁻¹ ampicillin and 35 μg mL⁻¹ chloramphenicol. Cells were grown at 37 °C to an OD₆₀₀ of 0.8, at which point the temperature was decreased to 18 °C for ~30 minutes. Protein expression was induced by addition of isopropyl-β-D-thiogalactoside.
DNA, and using HKL200034. The crystal structure of the 1nt-gapped SSB ternary complex was determined by X-ray crystallography. The crystal structure solution and refinement statistics are listed in Table1. Ramachandran 

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Competing interests
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