Characterization of SpPol4, a unique X-family DNA polymerase in Schizosaccharomyces pombe

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ABSTRACT

As predicted by the amino acid sequence, the purified protein coded by Schizosaccharomyces pombe SPAC2F7.06c is a DNA polymerase (SpPol4) whose biochemical properties resemble those of other X family (PolX) members. Thus, this new PolX is template-dependent, polymerizes in a distributive manner, lacks a detectable 3′→5′ proofreading activity and its preferred substrates are small gaps with a 5′-phosphate group. Similarly to Polμ, SpPol4 can incorporate a ribonucleotide (rNTP) into a primer DNA. However, it is not responsible for the 1–2 rNTPs proposed to be present at the mating-type locus and those necessary for mating-type switching. Unlike Polμ, SpPol4 lacks terminal deoxynucleotidyldtransferase activity and realigns the primer terminus to alternative template bases only under certain sequence contexts and, therefore, it is less error-prone than Polμ. Nonetheless, the biochemical properties of this gap-filling DNA polymerase are suitable for a possible role of SpPol4 in non-homologous end-joining. Unexpectedly based on sequence analysis, SpPol4 has deoxyribose phosphate lyase activity like Polβ and Polα, and unlike Polμ, suggesting also a role of this enzyme in base excision repair. Therefore, SpPol4 is a unique enzyme whose enzymatic properties are hybrid of those described for mammalian Polβ, Polα and Polμ.

INTRODUCTION

Efficient DNA repair is essential to maintain genome stability and cell viability (1,2). In spite of a variety of DNA repair mechanisms, one common step is DNA synthesis, carried out by specialized DNA polymerases. DNA polymerases are classified into four different groups according to their biochemical properties and to the biological processes in which they are involved. Among them, only family X DNA polymerases (PolX) are devoted to DNA repair, being evolutionarily conserved in prokaryotes, eukaryotes and archaea (3–5). However, their number ranges from five members in mammals [Polβ, Polλ, Polμ, terminal deoxynucleotidyldtransferase (TdT) and Polσ] to one member in yeasts, plants, and some bacteria and viruses, i.e. Saccharomyces cerevisiae (ScPol4), Arabidopsis thaliana (AthPolX), Bacillus subtilis (BsPol4X) and African swine fever virus (ASFVPolX). Interestingly, two model organisms, Caenorhabditis elegans and Drosophila melanogaster, whose genomes have been completely sequenced, have no putative PolX (3).

PolX enzymes most probably share a common modular organization (Polβ core) consisting of an 8 kDa domain and a 31 kDa polymerization domain comprising ‘fingers’, ‘palm’ and ‘thumb’ subdomains. Such a structural organization has been demonstrated for Polβ (6,7), TdT (8), Polλ (9,10) and ASFVPoI (11,12). Unlike Polβ, ASFVPoI, bacterial and archaea PolX members, other family enzymes (Polα, Polμ, TdT and ScPol4) have an additional domain, the Brca1 C-terminal, named BRCT, which has been suggested to take part in protein–protein and protein–DNA interactions (13). Besides this BRCT domain, Polλ AthPolX and ScPol4 have a proline-serine-rich region in their central part with a yet unknown function (3).

Regarding their biochemical properties, all DNA polymerases from this family are single-subunit enzymes, lacking the 3′→5′ exonuclease activity and displaying very low processivity during primer extension reactions [reviewed in (14)]. Polβ, the paradigm of the PolX family, inserts nucleotides in a template-dependent manner and is moderately accurate (15,16). Its preference for small gaps with a 5′-phosphate group (17) and its deoxyribose phosphate (dRP) lyase activity that relies on the 8 kDa domain (18) are properties consistent with a role in base excision repair (BER), a major pathway...
involved in the repair of damaged nucleotides (19,20). This multistep process is initiated with the removal of the modified base by a specific DNA N-glycosylase yielding apurinic/apyrimidinic (AP) sites. AP sites are recognized and incised by an AP endonuclease leaving single strand breaks (SSBs) with a 5′-dRP end. Finally, the 5′-dRP can be released either by the 5′-dRPase activity of Polβ (short-patch BER) or by the combined action of a DNA polymerase (Polβ, and Polσ or Polδ) and the 5′-flap endonuclease FEN1 (long-patch BER) (21,22).

Polλ has 32% amino acid identity to Polβ and contains an intrinsic dRP lyase activity that can substitute for Polβ in BER in vivo and in vitro (23,24). However, the high affinity of Polλ for deoxynucleotides (dNTPs) (37-fold over Polλ) has an essential role for Polλ in BER (30,31). Polλ is able to insert ribonucleotides (rNTPs) to a DNA chain (36,37) and lacks dRP lyase activity (25). In addition, immunodepletion of nuclear extracts of HeLa cells for deoxynucleotides (dNTPs) (37-fold over Polλ) has been reported in non-homologous end-joining (NHEJ). Polλ has 41% identity to TdT, a template-independent DNA Pol X responsible for the N-addition during V(D)J recombination of the immunoglobulin genes and T-cell receptor genes (30,31). Polλ-deficient mice are impaired in V(D)J recombination of the immunoglobulin κ light chain (32), which is initiated by an initiated double strand break (DSB) that is repaired by an NHEJ mechanism, similar to that employed by other tissues to repair DSBs. However, unlike TdT, whose expression pattern is restricted to lymphoid tissues, Polλ is expressed in additional tissues (33), suggesting a more general role of Polλ in DNA repair (34).

Polλ behaves as an error-prone DNA polymerase, since it is able to induce/accept dislocations of the template strand (35). Unlike Polβ and Polλ, Polσ is able to insert ribonucleotides (rNTPs) to a DNA chain (36,37) and lacks dRP lyase activity (24). Based on these properties and on the physical and functional interactions with the Ku-XRCC4–DNA ligase IV–DNA complex (27,29), Polλ is not a strictly template-dependent DNA polymerase, since it has an intrinsic terminal-transferase activity (33) that probably plays a role in microhomology-mediated NHEJ reactions (R. J. F. Ruiz, S. A. Nick McElhinny, D. A. Ramsden and L. Blanco, manuscript submitted).

In contrast to mammals, budding and fission yeasts have only one DNA PolX enzyme (3). Whereas ScPol4 is closely related to Polλ (25% amino acid identity to the Polλ core), the putative DNA PolX enzyme from the fission yeast Schizosaccharomyces pombe (SPAC2F7.06c) is more closely related to Polλ than to Polλ (27% versus 24% identical core residues, respectively). ScPol4 was the first DNA PolX shown to play a role in NHEJ (39). In agreement with that, it has been shown to have a direct interaction of the BRCT domain of ScPol4 with the Lig4/Lif1 complex (40), and a physical and functional interaction of Rad27 with both ScPol4 and Dnl4/Lif1 (41). No functional data have been reported for the putative DNA PolX (SPAC2F7.06c) from the fission yeast S.pombe, a unicellular eukaryotic organism whose properties closely resemble those of higher eukaryotic organisms. For this reason, S.pombe is a good model system for the analysis of gene products involved in DNA repair. Here, we report the cloning, expression and biochemical characterization of the SPAC2F7.06c gene product from S.pombe. DNA polymerization properties and the presence of a dRP lyase activity support a role of this DNA polymerase in both NHEJ and BER reactions. In spite of the closer similarity to Polλ, this enzyme combines Polβ, Polλ and Polσ properties, and therefore, it should be more unambiguously referred to as SpPol4.

MATERIALS AND METHODS

Strains and growth conditions

Cells were grown at 30°C in rich medium (YES; 0.5% yeast extract, 3% glucose and supplemented with 200 mg/l of leucine and uracil) or in minimal medium (EMM). Appropriate amino acids and thiamine were added to EMM when required to a final concentration of 200 mg/l and 25 μM, respectively. Geneticin selection was performed using YES medium containing 100 mg/l G418 (Sigma). The pol4Δ::KanMX strains, sp8 and sp10, were created from the wild-type strains, sp7 (h- leu1-32 ura4D18) and sp698 (h90), respectively, using the PCR-based method and the primers, 5′-TTCCCCATTGTTGAATTCTC-3′ and 5′-AGTAATGTGGCGATCTTAAAGGTCACAAGATCACAGTGCGCATCTGATATCATC-3′ and 5′-AGTAACTATTAGTTGATTGCAAGATCGTGAAGCTTCGTACGCT-3′ and 5′-CGTCAAGTTGGAATGCTCTAAGTGATTATCTGATATCATC-3′. The nucleotide sequences in boldface overlap to the KanMX cassette of plasmid pFA6a-kanMX4 (42). The deletion was confirmed by PCR using primers pol4.C (5′-ATCTGCTGTCAAAATGAAGATT-CTTGC-3′) and pol4.D (5′-CTGCAAGTTGGAATGCTCTAAGTGATTATCTGATATCATC-3′) and by Southern blot (data not shown).

Nucleotides and proteins

Ultrapure unlabeled dNTPs and rNTPs, [γ-32P]ATP, [α-32P]dTTP and [α-32P]ddATP (3000 Ci/mmol) were purchased from Amersham Biosciences. T4 polynucleotide kinase, UDG and T4 DNA ligase were purchased from New England Biolabs; TdT was obtained from Promega; restriction endonucleases and Taq Expand High Fidelity were obtained from Roche; HAP was a gift from Dr S. H. Wilson (NIEHS, Research Triangle Park, NC). Purified human Polλ and Polσ were obtained as described previously (25,33).

Oligonucleotides, templates and substrates for DNA polymerization

Synthetic DNA oligonucleotides were obtained from Invitrogen (P15, 5′-TCTGTGCAGGGTCTT-3′; P15 (C), 5′-TCTGTGCAAGGTGCCT-3′; SPIC, 5′-GATCACAGTTGATAC-3′; F6, 5′-CTGCACTGATGCCGUTAGCGTGACCCGGTAC-3′; T32 (A), 5′-TGAATCCCTTCGACAAAGAACCTCCTACAGAC-3′; T32 (C), 5′-TGAATCCCTTCGACAAAGAACCTCCTACAGAC-3′; T32 (G), 5′-TGAAGTTTACCTAGAGCGTACAGAGAC-3′; T32 (T),...
Cloning and purification of \textit{S. pombe} DNA polymerase X

Cloning of the \textit{S. pombe} \textit{Spol4} gene was started from the identification of an open reading frame (ORF) (SPAC2F7.06c), in the public database \textit{S. pombe}/GeneDB (http://www.genedb.org/) that codifies for a putative DNA polymerase from the \textit{X} family. Specific primers with restriction sites (in boldface) in their 5' ends \textit{Spol4} 5'\textit{BxG} (5'-AGATCTTGCTCGACGACTAAGATCTTGCTCAAG-3') and \textit{Spol4} 5'\textit{BbG} (5'-AGATCTGGCGCCCGCCTATCCCGTGTAGCAGAACAAG-3') were designed to amplify yeast genomic DNA. All the oligonucleotides were purified by PAGE with a 20% urea–20% PAGE gel. Oligonucleotides SP1C, P15 (C), P15, P6 and oligo(dT)15 were 5'-labeled with [γ-32P]ATP and T4 polynucleotide kinase. For dRP lyase assay, oligonucleotide P6 was 3'-labeled with [α-32P]dATP and TdT. Polymerase activity was evaluated by using synthetic double-stranded oligonucleotides as substrates. These substrates were prepared by annealing a 5'-3' end-labeled primer to different oligonucleotides to generate open (P15/T32 or SP1C/T18(T) and 1 or 2 nt gapped (P15/T32/D16; SP1C/T13(C)/DG1 or P15/T32/D15) template/primer substrates in the presence of 0.2 M NaCl and 60 mM Tris–HCl, pH 7.5. The polymerization reactions were done in 12.5 µl of a mixture containing 50 mM Tris–HCl, pH 7.5, 2 mM MgCl2, and 0.1 mg/ml BSA, 0.1% (w/v) xylene cyanol and 0.1% (w/v) bromophenol blue. Products were resolved and analyzed by denaturing 8 M urea–20% PAGE and autoradiography.

Quantification was done in a Fujix BAS1000.

Construction and purification of a polymerization-deficient form of \textit{Spol4} DNA polymerase

Site-directed mutations were introduced into pGEM-T Easy::Spol4 plasmid by using a PCR-based method (QuickChange® Site-Directed Mutagenesis kit; Stratagene) with the oligonucleotide 5'-GCCGTGGAGCCCGGGTT-GCTATGGTTGTAGTCC-3' and its reverse complementary oligomer (5'-GGGCTGGAGCAGCCCTT-3') and its reverse complementary oligomer. Site-directed mutations were introduced into pGEM-T Easy::Spol4 plasmid by using a PCR-based method (QuickChange® Site-Directed Mutagenesis kit; Stratagene). The incubation mixture contained, in 25 µl of 50 mM Tris–HCl, pH 7.5, 1 mM EDTA, 0.1% (w/v) xylene cyanol and 0.1% (w/v) bromophenol blue.

DNase I digestion and DNA polymerase assay

DNase I digestion and DNA polymerase assay were carried out as follows: 3.5 µl of 100 mM EDTA, pH 8, 1% NP-40, 1.3 mM p-nitrophenylbenzenamine, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 tablet of protease inhibitor cocktail (Roche) and broken with a French Press (twice at 20 000 psi). KCl was added to the lysate up to a final concentration of 0.2 M. Cell debris were separated from the soluble lysates by ultracentrifugation (50 000 g for 1 h at 4°C in a Beckman JA-25.50 rotor). The protein from the soluble fraction was subjected to affinity chromatography. Glutathione–Sepharose 4B (1 ml) (Pharmacia) packed into a column was equilibrated with 10 vol of lysis buffer. Soluble lysate was then loaded at 3 ml/h flow rate into the column. Afterwards, the column was extensively washed with buffer IPPI50 (PBS and 0.1% NP-40) and equilibrated with native binding buffer (100 mM Tris–HCl, pH 8 and 100 mM NaCl). After several washing steps with native binding buffer containing 20 mM glutathione, polymerase-containing fractions estimated by Coomassie blue staining were collected, 2-fold diluted with buffer A (50 mM Tris–HCl, pH 7.5, 10% glycerol, 0.5 mM EDTA and 1 mM DTT), and bound to a phosphocellulose column (1 ml) and eluted with buffer A containing 500 mM NaCl. This fraction contains highly purified GST-tagged \textit{Spol4} protein. Protein concentration was estimated by densitometry of Coomassie blue-stained 10% SDS–PAGE gels, using standards of known concentration. Under these conditions, the yield was 26 µg of purified GST-tagged \textit{Spol4} protein. This purified final fraction, adjusted to 50% (v/v) glycerol and supplemented with 0.1 mg/ml BSA, was stored at 70°C.

DNA polymerization on activated DNA

The incubation mixture contained, in 25 µl, 50 mM Tris–HCl, pH 7.5, 1 mM EDTA, 4% glycerol, 0.1 mg/ml BSA, 13.2 nM (α-32P]dCTP, 1 µM (dTTP, dCTP, dGTP, dATP), 1 mM MnCl2 or 10 mM MgCl2 as metal activator, 625 ng of activated calf thymus DNA and 250 nM of the purified GST-tagged \textit{Spol4} or \textit{Spol4} D355A/D357A. After incubation for 30 min at 37°C, the reactions were stopped by adding 10 mM TE/0.1% SDS and the samples were filtered through Sephadex G-50 spin columns in 10 mM TE/0.1% SDS. The excluded volume, corresponding to the labeled DNA, was counted (Liquid Scintillation Counter; Pharmacia) and the
polymerization activity of SpPol4 was calculated as the amount of incorporated dCMP.

3′→5′ exonuclease assay

The incubation mixture, in 12.5 µl, contained 50 mM Tris–HCl, pH 7.5, 10 mM MgCl₂, 1 mM DTT, 4% glycerol, 0.1 mg/ml BSA, 50 nM SpPol4 and 1.5 nM single-stranded labeled P15 or P15/T32(C) hybrid. Reactions were incubated at 30°C for 15 min and were stopped by adding denaturing loading buffer. 3′→5′ exonuclease activity expected to produce a degradation ladder of the labeled P15 primer, was analyzed by 8 M urea–20% PAGE and autoradiography.

Electrophoretic mobility shift assay (EMSA)

EMSA was performed using 1 [SP1C/T13(C)/DG1] and 5 nt [SP1C/T18(T)/DG5] gapped molecules to analyze the interaction of SpPol4 and DNA. Gel mobility shift assays were performed in a final volume of 12.5 µl containing 50 mM Tris–HCl, pH 7.5, 0.1 mg/ml BSA, 1 mM DTT, 4% glycerol, 4 mM labeled DNA and different concentrations of SpPol4 (250, 600 and 1200 nM). Samples were incubated for 10 min at 30°C to allow the formation of enzyme–DNA complexes. For competition analysis, 100 nM SpPol4 was incubated with labeled 1 nt gapped 5′-phosphate molecules (4 nM) for 15 min at 30°C in a final volume of 25 µl. After the complexes were formed, unlabeled 1 nt gapped 5′-phosphate (200 and 600 nM) or 1 nt gapped 5′-hydroxyl (200 and 600 nM) molecules were added to the reaction mixture and incubated for another 10 min at 30°C. After incubation, samples were mixed with 3 µl of 30% glycerol and resolved by native gel electrophoresis on a 4% polyacrylamide gel (80:1 monomer/dimer) containing 30% glycerol and resolved by native gel electrophoresis on a 4% polyacrylamide gel (80:1 monomer/dimer). For autoradiography, DNA polymerase–DNA complexes were detected as mobility retardation in the migration position of the labeled free DNA. Quantification of the competition experiments was done in a FujiX BAS1000. The amount of the labeled GAP1-P forming SpPol4::GAP1-P complexes was calculated by normalizing the radioactive signal in the shifted band to the total radioactivity.

dRP lyase activity assay

As a substrate, the 3′ end 34mer-labeled P6 oligonucleotide was annealed to the 34mer T4 oligonucleotide. This labeled double-stranded substrate (500 nM) was treated with UDG (100 nM) for 20 min at 37°C in buffer containing 50 mM HEPES, pH 7.5, 20 mM KCl and 2 mM DTT to remove the uracil. After incubation, the mixture was supplemented with 10 mM MgCl₂ and 40 nM APETase for 10 min at 37°C; thus, generating the substrate for dRP lyase activity. Reaction mixtures (25 µl) containing 50 mM HEPES, pH 7.5, 10 mM MgCl₂, 20 mM KCl, 2 mM DTT, 70 nM concentration of the treated substrate and different amounts of either SpPol4 (20, 60 and 120 nM), SpPol4[Δ555A/Δ375A] (20, 60 and 120 nM), hPolµ (70 nM) or hPolλ (60 nM) were incubated at 37°C for 20 min. After incubation, NaBH₄ was added to a final concentration of 340 mM, and the reactions were kept for 20 min on ice. Stabilized (reduced) DNA products were ethanol precipitated in the presence of 0.1 g/ml of tRNA, resuspended in water and analyzed with 8 M urea–20% PAGE and visualized by autoradiography.

In vitro reconstitution of BER

A 34mer double-stranded DNA substrate that contained a G opposite uracil at position 16 was used. This molecule was treated as described above for the dRP lyase activity assay to generate the dRP-containing substrate. Reactions (25 µl) containing 70 nM substrate, 50 mM HEPES, pH 7.5, 10 mM MgCl₂, 2 mM KCl, 2 mM DTT, 0.3 µM (α-32P)dCTP and either SpPol4 (125 nM) or human Polλ (245 nM) were incubated for 20 min at 37°C. Later, each reaction was divided into two halves. One was incubated with 1 mM ATP and 40 U T4 DNA ligase for 10 min at 37°C and the other was mock-treated. Reactions were terminated by the addition of denaturing loading buffer, analyzed by 8 M urea–20% PAGE and visualized by autoradiography.

Genomic DNA preparation and imprint analysis in S.pombe

Yeast chromosomal DNA was purified from a logarithmically growing culture (10 ml; OD600 ~0.5–1). Cells were harvested and resuspended in 200 µl breaking buffer (2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris–HCl, pH 8 and 1 mM EDTA), ~200 µl glass beads and 200 µl phenol/chloroform/isoamyl alcohol (25:24:1). After 15 min vortexing at high speed, 200 µl TE was added and the mixture was centrifuged for 5 min at 11000 g. The aqueous layer was transferred to a clean tube and DNA was precipitated with ethanol. The pellet was resuspended in TE. HindIII-digested DNA (50 µg) was separated by agarose gel electrophoresis and analyzed by Southern hybridization using a 1 kb 32P-labelled mat1-P PCR probe. The oligonucleotides sequences used for the PCR were mat1-5′, 5′-AGAAGAGAGATGTTGAGG-3′; and mat1P-3′, 5′-CCAAATCTCTTTGATATGTTATAC-3′. The mat2 (6.3 kb) and mat3 (4.2 kb) bands result from hybridization to the mat1 probe as they share cassette homology. The imprint could be converted into a DSB during standard methods of DNA purification (43,44) and visualized by autoradiography. To determine the efficiency of mating-type switching, a standard iodine staining assay was carried out. Individual colonies were replicated onto EMM supplemented with the appropriate amino acids and then were grown for 3 days at 22°C before being exposed to iodine vapors.

RESULTS

SpPol4 a unique X-family DNA polymerase in S.pombe

The S.pombe ORF SPAC2F7.06c coding for a putative 736 amino acids DNA polymerase from the X family was overproduced in fission yeast wild-type cells and purified to near homogeneity as described in Materials and Methods. The protein, expressed as a fusion protein containing a GST-tag at its N-terminus, was purified by glutathione–Sepharose affinity and phosphocellulose chromatography. After the purification steps a unique polypeptide was observed in the final fraction, identified by Coomassie blue staining after SDS–PAGE analysis, migrating at the expected position for GST-tagged SPAC2F7.06c (~84 kDa) (data not shown). The purified fraction was assayed for DNA polymerase activity on an activated DNA. As expected, the purified fraction was able to catalyze dNTP incorporation in the presence of either
with a fully distributive polymerization pattern. This distribu-
tion decreased with the enzyme/DNA substrate ratio in agreement
in Supplementary Figure 1, the length of the elongated primer
distribution at several enzyme/DNA substrate ratios. As shown
DNA template/primer substrate by analyzing the chain length
(16, 25, 35, 36, 45, 46). We assessed
Sp
mon feature of all DNA polymerases from the X family
nucleotides more slowly and consequently synthesize DNA
sequently display weaker DNA interactions and incorporate
nucleotide insertion. Conversely, DNA repair enzymes fre-
merases), and relies on a tight DNA binding and an efficient
involved in extensive DNA synthesis (i.e. replicative poly-
Processivity is a common feature of DNA polymerases
15 min at 30

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Group was present at the 5
0
polymerization capacity was observed when a phosphate
substrates in a dNTP dosage-dependent manner (Figure 1A and B).
However, a significant increase (10-fold as an average) in the
substrate preferences of SpPol4. (A) Scheme of the two types of DNA molecules used: 1 nt gap (GAP1-OH) and
1 nt gap with a 5'-phosphate (GAP1-P). Labeled primers (asterisk) and the 5'
end hydroxyl (OH) or phosphate (P) groups are indicated. The oligonucleotides
used were T13(C)/SP1C/DG1. (B) Gap-filling DNA synthesis of SpPol4 on the
1 nt gapped structures described above. Reactions were carried out as described
in Materials and Methods using 125 nM SpPol4 and the indicated concentra-
tions of dGTP. Primer extension was analyzed by 8 M urea–PAGE and auto-
radiography. Mobility of the unextended primer (P) and the 1 nt (1+) extended
primers are indicated at the autoradiograph. (C) DNA-binding capacity of
SpPol4 to the 1 nt gapped molecules. EMSA was performed as described in
Materials and Methods using none (lanes 1 and 5), 250 nM (lanes 2 and 6),
600 nM (lanes 3 and 7) and 1.2 µM (lanes 4 and 8) SpPol4. Formation of
SpPol4:DNA complexes was resolved by native gel electrophoresis on a 4%
polyacrylamide gel (80:1; monomer/bis). Mobility of the free DNA (F) and the
SpPol4:DNA complexes (C) are indicated at the autoradiograph. (D) Competi-
tion analysis of SpPol4 bound to the GAP1-P. EMSA was performed as de-
scribed in Materials and Methods, but using the indicated amounts of either 1 nt
gapped 5'-phosphate (G1-P) or 5'-hydroxyl (G1-OH) as unlabeled competitor
cDNA. The plotted values represent the percentage of labeled GAP1-P that
remains bound to SpPol4 after competition, and are the mean of four independ-
ent experiments.

contains an N-terminal 8 kDa domain, and is stimulated by the
presence of a 5'-phosphate group in the 1 nt gapped substrate,
we tested if this stimulation was primarily due to differences
in the DNA-binding capacity, a step preceding dNTP binding


Figure 1. Gap-filling synthesis and substrate preferences of SpPol4. (A) Scheme of the two types of DNA molecules used: 1 nt gap (GAP1-OH) and
1 nt gap with a 5'-phosphate (GAP1-P). Labeled primers (asterisk) and the 5'
end hydroxyl (OH) or phosphate (P) groups are indicated. The oligonucleotides
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remains bound to SpPol4 after competition, and are the mean of four independ-
ent experiments.

SpPol4 is a distributive polymerase that lacks
3'→ 5' exonuclease activity

Processivity is a common feature of DNA polymerases
involved in extensive DNA synthesis (i.e. replicative poly-
erases), and relies on a tight DNA binding and an efficient
nucleotide insertion. Conversely, DNA repair enzymes fre-
ditionally display weaker DNA interactions and incorporate
nucleotides more slowly and consequently synthesize DNA
in a distributive mode. Distributive polymerization is a com-
mon feature of all DNA polymerases from the X family
(16, 25, 35, 36, 45, 46). We assessed SpPol4 processivity on a
DNA template/primer substrate by analyzing the chain length
distribution at several enzyme/DNA substrate ratios. As shown
in Supplementary Figure 1, the length of the elongated primer
distribution decreased with the enzyme/DNA substrate ratio in agreement
with a fully distributive polymerization pattern. This distribu-
tive behavior of SpPol4 is also maintained using Mn

SpPol4 prefers small gaps with a 5'-phosphate group

To further characterize the DNA polymerization activity pre-
ent in the purified SpPol4 fraction, we tested different in vitro
conditions using defined templated-DNA molecules in the
presence of Mg

2+ (2 × 10

−5 pmol/min ng) or Mn

2+ (9 × 10

−6 pmol/min ng) as activating divalent metal ions. As a control of specificity,
we carried out a parallel purification of a catalytically inactive mutant (see Materials and Methods). In this case, no DNA
polymerization activity was detectable in the final fraction
(data not shown). Therefore, SPAC2F7.06c codifies for a
DNA polymerase that we refer to as SpPol4.

Another distinctive feature of replicative DNA polymerases
is its proofreading 3'→5' exonuclease activity. Three con-
served amino acid motifs, named Exo I, Exo II and Exo III,
are responsible for the 3'→5' exonuclease active site of all
proofreading DNA polymerases (47). However, these motifs
are absent in the DNA polymerases from the X family includ-
ing SpPol4, suggesting that, as other PolX enzymes, SpPol4
has no proofreading activity. We tested this prediction using
either a single-stranded oligonucleotide or a template/primer
as substrates for 3'→5' exonucleolysis. Purified SpPol4 failed
to display any nucleolytic activity on both substrates after
15 min at 30°C (data not shown). This result demonstrates
that SpPol4 does not possess 3'→5' proofreading activity.

In Polβ and Polλ a 5'-phosphate-dependent increase in pro-
cessivity is structurally and functionally related to the presence
of the N-terminal 8 kDa domain (5, 7, 9, 48). Since SpPol4 also
and catalysis. The formation of stable SpPol4/DNA complexes, assessed by EMSA, required a lower enzyme concentration when the 1 nt gapped DNA had a 5'-phosphate group (Figure 1C). Even more, the affinity of SpPol4 for the 5'-phosphate group is so strong that when the primer strand is removed, SpPol4 still binds this molecule almost with the same efficiency (data not shown).

To further analyze the stabilizing effect of the 5'-phosphate on DNA binding by SpPol4, competition analysis were carried out as indicated in Materials and Methods. As expected, the amount of SpPol4::GAP1-P (labeled) complexes formed in a previous step progressively decreased when increasing amounts of unlabeled competitor DNA (either having a 5'-phosphate or not) were added, being greater the competition with unlabeled GAP1-P (Figure 1D). However, even at 150-fold molar excess of the 1 nt gapped 5'-phosphate competitor, the amount of SpPol4::GAP1-P labeled complexes was reduced only by 5% in comparison with that in the absence of the competitor (Figure 1D). Taken together, these results clearly demonstrate that SpPol4 binds stably and preferentially to a 5'-phosphate containing DNA gap.

SpPol4 is a template-instructed polymerase with preference for purines

We evaluated the ability of SpPol4 to discriminate among the four dNTPs in order to catalyze template-directed DNA synthesis. We used a set of 1 nt gapped template-primer substrates with each of the four (X = A, C, G or T) bases as template and having a 5'-phosphate flanking the gap (Figure 2A). For each substrate, the four dNTPs, one complementary to the template and the other three non-complementary, were assayed individually at different concentrations. As shown in Figure 2B, on the four 1 nt gapped substrates, SpPol4 preferentially incorporated the nucleotide complementary to the first template base, even when non-complementary nucleotides were provided at a 100-fold higher concentration. Therefore, these results suggest that SpPol4 is template-instructed, i.e. it performs DNA synthesis following the Watson–Crick base pairing rules. Interestingly, quantification of the efficiency of incorporation of each complementary dNTP demonstrated a strong imbalance in correct dNTP incorporation with preference for purines: dG>dA>dT>dC (Figure 2C).

Template dislocation and primer realignment capacities of SpPol4

Human Polθ behaves as an error-prone DNA polymerase, since it is able to induce/acceot dislocations of the template strand (35), which is likely crucial for its NHEJ function. To examine whether SpPol4 is similarly error-prone, we analyzed DNA synthesis in some template sequence contexts that are appropriate for evaluating: (i) slippage-mediated dislocation (Figure 3A); (ii) dNTP selection-mediated dislocation (Figure 3B); and (iii) primer realignment versus direct mismatch extension (Figure 3C). Each dNTP was provided individually to identify the opted mechanism for each DNA polymerase. In the substrate with the dA-track repeat, normal DNA synthesis would lead to dT incorporation, whereas slippage of the primer terminus (dT) to the next template base (dA) would result in dG incorporation and a −1 frameshift DNA synthesis (see schematic representation in Figure 3A).

Unlike Polθ, which clearly preferred to insert dG by a slippage-mediated dislocation mechanism, SpPol4 and Polλ predominantly incorporated dT (Figure 3A). However, both polymerases also incorporated dG and therefore, they can misalign the template-primer to some extent. As shown in Figure 3B, changing the third dA of the track for a dG reduces the possibility of template slippage; therefore, DNA synthesis is more restricted to the canonical incorporation of dC, like SpPol4 and Polλ do. Besides this normal DNA incorporation event, only Polθ was also able to insert the complementary base (dG) to the position +2 in the template (dC). As reported earlier for Polθ, the template dislocation requirement would be stabilized by the incoming dNTP (49).

To examine the mismatch extension capacity of SpPol4, we performed a primer extension assay starting from a dA:dC base pair mismatch (see schematic representation in Figure 3C). Because SpPol4 and other PolX enzymes cannot remove mismatched nucleotides at the primer 3' end, only two outcomes are possible: (i) direct mismatch extension inserting dC; and (ii) primer terminus realignment inserting dG. As can be seen in Figure 3C, Polλ uses both alternatives almost equally well. In agreement with its extreme error-proneness, Polθ is able to extend the mismatch with any of the four dNTPs. Interestingly, SpPol4 has a more restricted behavior, as it is only able to insert dG, indicating a significant primer realignment capacity that enables this enzyme for a role in NHEJ.
SpPol4 inserts both rNTPs and dNTPs with the same efficiency

Polμ and TdT have the striking ability to incorporate both rNTPs and dNTPs to nucleic acid chains (36,37,45). This unusual capacity mainly relies on a single glycine residue that opens the 'steric gate', which is frequently closed by a conserved aromatic residue present in Polβ, Polλ, and in other members of the PolX family of DNA-dependent DNA polymerases (Figure 4A) (36). Since SpPol4 also has a glycine residue at this position (Gly345), it seemed very likely that SpPol4 could incorporate rNTPs. By using the same four 1 nt gapped template–primer substrates as in Figure 2B, it was shown that SpPol4 efficiently incorporates rNTPs on a DNA primer strand (Figure 4B) with almost equal efficiency as dNTPs and displaying the same preference pattern for purines (compare Figures 4B and 2C).

Using a competition assay in which both sugars (ribose and deoxyribose) are simultaneously provided, the sugar selectivity factor of a given DNA polymerase can be calculated (36,45). Since the rNTP and the dNTP have different molecular weights, the +1 extended primers can be easily separated by gel electrophoresis and quantified. The sugar selectivity factor is given as the ratio between the amounts of primer extended with rNTP versus dNTP. Irrespective of the nature of the base, the sugar selectivity factors obtained for SpPol4 were very similar (0.74–0.91) and proximal to one, indicating a lack of discrimination between rNTPs and dNTPs (Figure 4C). These values are similar to those obtained for TdT (43) and Polμ (36) in untemplated and templated reactions, respectively, being several orders of magnitude smaller than the ones reported for other DNA polymerases (50–52).

**SpPol4 is not required for imprinting at the mat1 locus**

It is known for many years that mating-type switching in *S.pombe* depends on a strand-specific imprint at the mat1 locus (53,54). The imprint was characterized either as an alkali-labile modification or as a nick that could be converted into a DSB during standard methods of DNA purification (43,44). More recently, this imprint has been characterized as an RNase-sensitive modification that consists of one or two RNA residues incorporated into the mat1 locus (55). Based on these results, it was tempting to speculate that SpPol4 might be responsible for the incorporation of these one or two RNA residues. *S.pombe* genomic DNA was prepared by a standard yeast extraction protocol (see Materials and Methods), digested with HindIII and analyzed by Southern hybridization using a 1 kb mat1-P as a probe. The h50 wild-type strain yielded the typical bands of uncleaved (10.4 kb; mat1*) and cleaved (5.4 kb; mat1*) products, together with two other bands representing cross-hybridization of the mat1-P probe with the mat2 (6.3 kb) and mat3 (4.2 kb) loci (Supplementary Figure 2A and B, lane 2). As expected, the smt-0 mutant strain (56), containing a deletion of the cis-acting elements SAS1 and SAS2 and thus preserving the integrity of the cleavage site sequence while abolishing mating-type switching, did not produce the mat1* band (57) (Supplementary Figure 2B, lane 1). However, the amount of cleavage at the mat1 locus, represented by the 5.4 kb (mat1*) band, obtained in the h50 pol4Δ mutant was the same as the level obtained in the h50 wild-type strain (Supplementary Figure 2B, compare lanes 2 and 3). Moreover, direct measurement of the mating-type switching efficiency by the iodine staining assay (see Materials and Methods) was carried out. The starch reaction with iodine vapors stains spore-containing colonies black, whereas slow-switching mutants exhibit streaky iodine staining patterns and colonies unable to switch to a different mating type remain white (58). Based on these results, it was shown that SpPol4 efficiently incorporates rNTPs on a DNA primer strand (Figure 4B) with almost equal efficiency as dNTPs and displaying the same preference pattern for purines (compare Figures 4B and 2C).
In the mating type, appear yellowish. As expected, iodine vapors stained the smt-0 mutant colonies yellowish, whereas the h90 wild-type and the pol4D colonies were stained black (data not shown). Therefore, the imprint at mat1 remains unaffected in the absence of SpPol4 and if there were one or two RNA residues in the DNA, the incorporation would be SpPol4-independent.

**SpPol4 has no TdT activity**

Polμ, as TdT, displays an intrinsic deoxynucleotidyltransferase activity, which is stronger in the presence of Mn2+ as cofactor (33). This enzymatic activity requires a region of the palm subdomain called loop1, which is absent in SpPol4, Polβ and Polλ (Supplementary Figure 3A). It has been demonstrated that the deletion of this loop abolishes the TdT-like activity of human Polμ (29) (R. Juarez, J. F. Ruiz, S. A. Nick McElhinny, D. A. Ramsden and L. Blanco, manuscript submitted). TdT activity can only be unambiguously determined by using single-stranded homopolymeric DNA as primer and any of the three dNTPs not included in the primer sequence. Thus, using a 32P-labeled 15mer dT oligonucleotide, in the presence of either Mg2+ or Mn2+, SpPol4 displayed no TdT activity (Supplementary Figure 3B).

**SpPol4 has an intrinsic dRP lyase activity most probably involved in BER**

The amino acid residues that are critical for dRPase activity are conserved in the 8 kDa domain of Polβ and Polλ (18,24) and are indicated with dots in Figure 5A. Among them, a specific lysine (Lys72 in Polβ and Lys312 in Polλ) is the catalytic residue acting as a Schiff-base during β-elimination of the dRP moiety. As shown in Figure 5A, Polμ and SpPol4 lack the catalytic lysine residue, and as it has been demonstrated for Polλ (24), it was probable that SpPol4 was devoid of dRP lyase activity. However, by using standard BER assays (18,24), here we show that SpPol4 is able to remove a dRP group and...
promote single-patch BER \textit{in vitro}. By adding UDG and hAPE to a uracil-containing substrate, a nicked strand with a 3-hydroxyl and a 5'-dRP is produced. The strand containing the 5'-dRP moiety, which is 3' end labeled, migrates at the expected position of an 18mer + dRP (Figure 5B). By using increasing amounts of SpPol4, this product was converted to a shorter product (18mer), indicating that SpPol4 as Pol\(\lambda\), and unlike Pol\(\beta\), has an intrinsic dRP lyase activity (Figure 5B).

Similarly, the SpPol4D355A/D357A polymerization-deficient mutant was proficient in dRP lyase activity similar to the wild-type SpPol4 (Figure 5B). Unexpectedly from the alignment shown in Figure 5A, SpPol4 has dRP lyase activity though it lacks the lysine residue conserved in Pol\(\beta\) (Lys72) and Pol\(\lambda\) (Lys312) responsible for their dRP lyase activities (24,58).

Removal of a dRP residue is an essential step for the completion of single nucleotide BER. Pol\(\beta\) and Pol\(\lambda\) are able to efficiently promote \textit{in vitro} BER of a uracil-containing duplex.
DNA in the presence of hUDG, hAPE and DNA ligase I (24,59,60). As shown in Figure 5C, two main products were observed in a human Polλ-based reconstituted BER reaction: a 16mer product generated by a single nucleotide insertion (dCTP labeled) at the 3'-hydroxyl end of the 5'-incised AP site, and a 34mer product that corresponds to the complete repair of the DNA strand upon DNA ligase action. As shown in Figure 5C, both SpPol4 and hPolλ are able to produce the same 16 and 34mer labeled products. Thus, SpPol4 is able to coordinate both the gap-filling and dRP excision steps of repair preceding DNA ligase action. These data are consistent with a role for SpPol4 in BER and predict that the dRP lyase-containing enzyme SpPol4 could participate in BER in vivo.

**DISCUSSION**

In mammals, there are five members belonging to the X family of DNA polymerases: Polβ, Polλ, Polμ, Polρ and TaqDNA Polymerase. Unlike the, yeasts, plants, and some bacteria and viruses have only one PolX enzyme (3). In *S. pombe*, the entry SPAC2F7.06c (GeneDB http://www.genedb.org/) predicted a putative DNA PolX as inferred by sequence comparison analysis. Based on the results presented here, it can be concluded that SPAC2F7.06c does code for a novel DNA polymerase belonging to the PolX family that would be appropriately designated as SpPol4.

The structural organization of SpPol4 as an N-terminal BRCT domain followed by a C-terminal 39 kDa Polβ-like core domain resembles members of the family X DNA polymerases, such as Polμ, TaqDNA Polymerase. Excluding the more variable N-terminal BRCT domain (61), SpPol4 is more closely related to Polμ (27% identical core residues), followed by Polλ (24% identity) and Polβ (20% identity). It is worth noting that budding yeast has also one DNA PolX (ScPol4) that, unlike SpPol4, resembles Polλ in its core domain (25% identity) and in its structural organization (62). Therefore, based only on their coding sequences, it was speculated that SpPol4 is a yeast orthologue of Polμ, whereas ScPol4 would be an orthologue of Polλ (3).

As summarized in Table 1, our biochemical analysis demonstrated that SpPol4 is capable of carrying out DNA synthesis in a template-dependent manner and exhibits low processivity during primer extension. Such properties are shared by all members of the eukaryotic X family, except TaqDNA Polymerase. EMSAs showed that SpPol4 binds to 5'-phosphate-gapped substrates better than to those with a 5'-hydroxyl, and this should imply an improvement in polymerization on the former substrates. As indicated in Table 1, the improved polymerization activity dependent on a 5'-phosphate group described here for SpPol4 is also an attribute of Polβ and Polλ (23), and Polμ (R. Juárez, P. Andrade and L. Blanco, unpublished data), but not of ScPolIV (64).

Most residues involved in dRP lyase activity are conserved between Polβ and Polλ (24). Among them, the nucleophile residue at position Lys-72 (Polβ) or Lys-507 (Polλ), responsible for 90% of the activity (18,24), is conserved in ScPol4 (Lys-234), but not in Polλ and TaqDNA Polymerase (which lack dRP lyase activity), and is also absent in SpPol4. Unexpectedly, SpPol4 was shown to have dRP lyase activity although it lacks this conserved residue. Nonetheless, other residues proposed in Polβ to facilitate removal of the dRP group are indeed present in SpPol4, and an alternative lysine (Lys-308) could be acting as the attacking nucleophile (for details see Figure 5). In any case, and based on our in vitro assays, we propose that SpPol4 could play a role in BER, as Polβ and Polλ. Based on the demonstration of an intrinsic dRP lyase activity, a similar role for ScPol4 has been proposed recently (64).

As shown in this paper, the relative nucleotide usage of SpPol4 is different from that observed for other DNA-dependent DNA polymerases of the X family (Polβ, Polλ and Polμ). In particular, SpPol4 preferentially inserts purine nucleotides in the following order: dG>dA>dT>dC. Hydrolysis, alkylation, oxidation and deamination are the major forms of DNA damage in all living cells, which are mainly repaired by BER. It is worth noting that, at least in mammalian cells, purines are lost 20-fold more frequently than pyrimidines (~10,000/cell/day versus ~500/cell/day, respectively). Additionally, purines are the most frequently alkylated bases and guanine is the base more prone to oxidation, resulting in 8-oxoG (100–1000/cell/day) and along with adenine in a ring-opened form called formamidopyrimidine (FaPyG and FaPyA). Only deamination, an another prevalent form of DNA damage, occurs predominately at cytosine, turning it into uracil (100–500/cell/hour) (1.21). Therefore, it is tempting to speculate that the preference of SpPol4 for purine nucleotides has been adapted to cope with a more intensive role of repairing purine bases.

In addition to its preference for small gaps, the unusual capacity of SpPol4 to accept misaligned template–primer molecules as a substrate and to realign 3'-terminal mismatches would be very convenient for microhomology-mediated NHEJ. Moreover, some BER intermediates, as staggered nicks made by an AP endonuclease in opposite strands, originate DSBs that would trigger the NHEJ pathway. Under these circumstances, a DNA repair polymerase endowed with dRP lyase activity would be very convenient to process the damaged DNA ends and eliminate the dRP residues.

**Table 1.** Comparison of SpPol4 properties to other template-dependent members of the DNA PolX family

<table>
<thead>
<tr>
<th>DNA PolX</th>
<th>Stimulation by 5'-P</th>
<th>Dislocation by slippage</th>
<th>Dislocation by dNTP selection</th>
<th>rNTP usage</th>
<th>Terminal transferase</th>
<th>dRP lyase</th>
</tr>
</thead>
<tbody>
<tr>
<td>SpPol4</td>
<td>Yes</td>
<td>Yes</td>
<td>weak</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>ScPol4</td>
<td>Yes</td>
<td>Yes</td>
<td>weak</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Poll</td>
<td>Yes</td>
<td>Strong</td>
<td>strong</td>
<td>Yes</td>
<td>No</td>
<td>weak</td>
</tr>
<tr>
<td>Polμ</td>
<td>Yes</td>
<td>Strong</td>
<td>weak</td>
<td>No</td>
<td>weak</td>
<td>Yes</td>
</tr>
<tr>
<td>Polβ</td>
<td>Yes</td>
<td>Very strong</td>
<td>strong</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Polλ</td>
<td>Yes</td>
<td>Strong</td>
<td>weak</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* Taken from (64).
depend on a steric barrier for the 2′-hydroxyl of an incoming rNTP (4,50,65). Accordingly, Polβ and Polδ are unable to incorporate rNTPs since they have bulky residues close to the 2′ position of the ribose of the incoming nucleotide (Thr561-Phe562 and Tyr305-Phe306, respectively). However, Polα and TdT, which efficiently insert rNTPs (36,37,45), have a small residue in the pair (Gly433-Trp434 and Gly448-Trp449) that was shown to be responsible for rNTP insertion (34). As shown here, SpPol4 resembles Polα and TdT, as it also incorporates rNTP very efficiently. This property was expected because the two residues equivalent to Polα (Gly433-Trp434) are strictly conserved in SpPol4 (Gly433-Trp434). Strikingly, it has been recently reported that ScPol4, although having two aromatic residue at these positions (His517-Tyr518) also incorporates rNTPs with a high efficiency (64).

It has been demonstrated that NHEJ is a predominant repair pathway in G1 phase and probably in non-cycling cells (66–68). In contrast to dNTPs, abundant during S phase, rNTPs are available at high levels in all phases of the cell cycle (69,70). Therefore, as suggested for human Polδ (38) and ScPol4 (64), the extraordinary ability of SpPol4 to incorporate rNTPs would be very convenient for a role in NHEJ. Moreover, insertion of rNTPs might also be useful in BER to repair modified or damaged bases into DNA throughout the mammalian genome. This is particularly true for PolX super-family, which is also consistent with a less dependence on a steric barrier for the 2′ position of the ribose of the incoming nucleotide (69,70). Therefore, as suggested for human Polβ (Tyr271-Phe272 and Tyr505-Phe506, respectively). However, Polα and TdT, as it also incorporates rNTP very efficiently. This property was expected because the two residues equivalent to Polα (Gly433-Trp434) are strictly conserved in SpPol4 (Gly433-Trp434). Strikingly, it has been recently reported that ScPol4, although having two aromatic residue at these positions (His517-Tyr518) also incorporates rNTPs with a high efficiency (64).

It has been reported that the imprinting step during mating-type switching in S. pombe is an RNase-sensitive modification that consists of one or two RNA residues incorporated into the mat1 locus (55), which becomes a fragile chromosome site. Taking into account the capacity of SpPol4 to incorporate a few rNTPs in the DNA, it was tempting to speculate with a Spmat1 locus type switching in S. pombe. As shown here, the lack of SpPol4 (h30 polΔ strain) did not affect either the level of DSBs in the mat1 locus or the mating-type efficiency with respect to the wild-type strain; however, we conclude that the imprint should remain unaffected. Thus, if there were some RNA residues in the mat1 locus, the incorporation would be SpPol4-independent. Alternatively, the imprint could imply a strand-specific nick with no flanking RNA residues (72).

In conclusion, the results presented here demonstrate that SpPol4 shares biochemical properties with different members of the PolX super-family; thus, it must be considered to be a unique enzyme (see Table 1 for a comparison). Mammalian PolXs became specialized to play a role in BER (Polβ), in NHEJ coupled with BER (Polδ), microhomology-mediated NHEJ (Polμ) or V(DJ) recombination (TdT). However, the fact that both fission and budding yeasts had only one DNA Pol X suggests that they are evolutionarily closer to the stem ancestor of the family, which is also consistent with a less specialized and multipotential role in different forms of DNA repair, enabled by a combination of the biochemical properties of their mammalian homologues.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at NAR Online.

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REFERENCES


a novel eukaryotic DNA polymerase with a potential role in meiosis.


