SUPPLEMENTAL INFORMATION

DNA Polymerases δ and ζ Switching by Sharing the Accessory Subunits of DNA Polymerase δ

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES.

Cloning of human DNA Pols constructs to pColaDuet-1 expression vector.

cDNAs for hPol δ p125, p50 and p66 subunits were obtained from Open Biosystems (Clone IDs 3634655, 2822169 and 40010009, respectively). A pASHSUL vector expressing the first 98 residues of *S. cerevisiae* SMT3 (SUMO) and pSUPER expressing SUMO-specific protease dtUD1 (doubly tagged UD1) were a generous gift from Patrick Loll (Drexel University). dtUD1 was expressed and purified as described in (1).

A full-length cDNA for the p66 subunit was made as described previously (2). The DNA sequence coding for 144 amino acids of the N-terminal p66 fragment ($p66_N$) was cloned into pCOLADuet-1 plasmid (Novagen) at *Nco*I/*Eco*RI restriction sites. A His_6-p50 encoding sequence was ligated at *Nde*I/*Xho*I into pCOLADuet-1 containing the $p66_N$ encoding sequence. The gene fragment, corresponding to p125 residues 1001-1107, was cloned to pET-28b at *Nco*I/*Bam*HI sites. Then a $p125_C$-encoding sequence with adjacent 61- and 25-bp non-coding regions at 5'- and 3'-termini, respectively, was amplified and cloned into pCOLADuet-1 containing DNA for $p66_N$ and p50 by using overlap extension PCR according to (3). The resulting construct contains a $p125_C$-encoding sequence (together with Shine-Dalgarno box) inserted after the p50 gene, which allows them to be expressed independently from dicistronic mRNA. To allow for ligation-dependent cloning of different DNA sequences coding for SUMO-CTDs in place of $p125_C$, the obtained plasmid was partially digested with *Nco*I and *Bam*HI and the appropriate fragment was purified by agarose gel.

The construct for SUMO-tagged $p125_C$ was generated by ligation-independent cloning of the corresponding gene fragment into the pASHSUL vector as described in (1). Then the SUMO-$p125_C$-encoding sequence (without a His-tag) was amplified and ligated to *Nco*I/*Bam*HI-digested pCOLADuet-1 containing sequences for $p66_N$ and p50. The gene fragment encoding for hRev3 (p353) residues 3028-3130 was amplified from pPGR3d-1/REV3L (a generous gift from P.E.M. Gibbs, Rochester University) and subcloned into pASHSUL as described above. Then the SUMO-$p353_C$-encoding sequence (without a His-tag) was amplified and ligated to *Nco*I/*Bam*HI-digested pCOLADuet-1 containing sequences for $p66_N$ and p50.

The gene fragment corresponding to p180 residues 1265-1462 was amplified from pcDNA3/POLA1 (a generous gift from Motoshi Suzuki, Kyoto University). The SUMO sequence was amplified from pASHSUL with an overlap region for $p180_C$ at the 3'-end. Then sequences for SUMO and $p180_C$ were combined together by applying the fusion PCR technique (4). After digestion with *Nco*I/*Bam*HI, the final PCR product was ligated to pCOLADuet-1 containing sequences for $p66_N$ and p50. The p70-encoding sequence was amplified from cDNA (Open Biosystems; clone ID 2822514) with chimeric primers containing the regions complementary to the vector sequence flanking the gene for p50. Finally we replaced the p50 gene with one encoding for p70 in pCOLADuet-1 containing the SUMO-$p180_C$ and $p66_N$ sequences by using the overlap extension PCR (3). The p59 sequence was amplified from cDNA (Open Biosystems; clone ID 8991936) and inserted by PCR instead of the p50 gene in pCOLADuet-1 containing SUMO-$p353_C$ and $p66_N$ sequences. Then we amplified the $p261_C$ gene fragment from pCR-XL/POLE1 (5) with chimeric primers containing the regions complementary to the vector sequence flanking the $p353_C$ gene. Finally the $p261_C$ sequence was inserted by overlap extension PCR directly after the SUMO sequence (in place of p353) into pCOLADuet-1 containing the p59 and $p66_N$ sequences.

We used multiple-site plasmid mutagenesis protocol (6) in order to replace cysteines 3042, 3045, 3054, 3057 for alanine at MBS1 and cysteines 3086, 3089, 3099, 3104 at MBS2 in pASHSUL/SUMO-$p353_C$.

All plasmid constructs used in this study were verified by full-length sequencing. Primers sequences are available upon request.

SUPPLEMENTAL FIGURES AND LEGENDS.

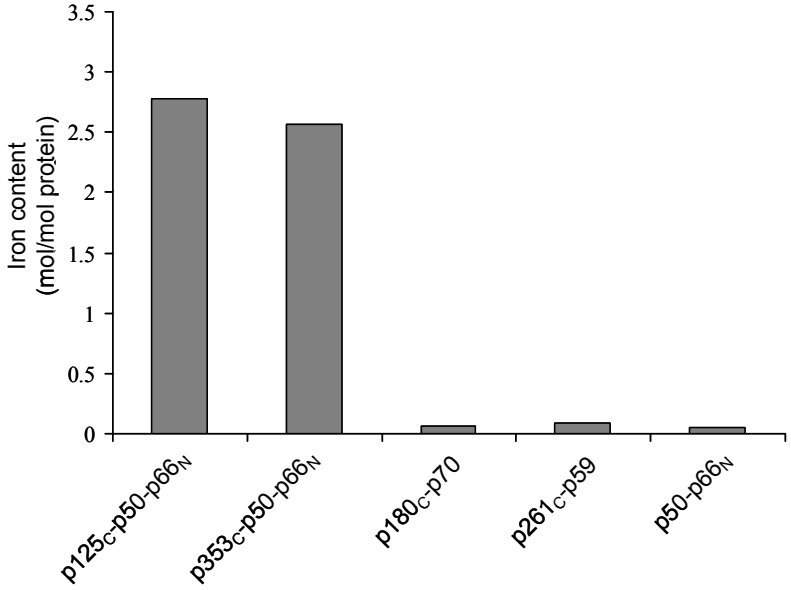

Figure S1. Iron content in purified human DNA Pol subcomplexes. Columns are an average of two independent experiments including protein expression and purification.

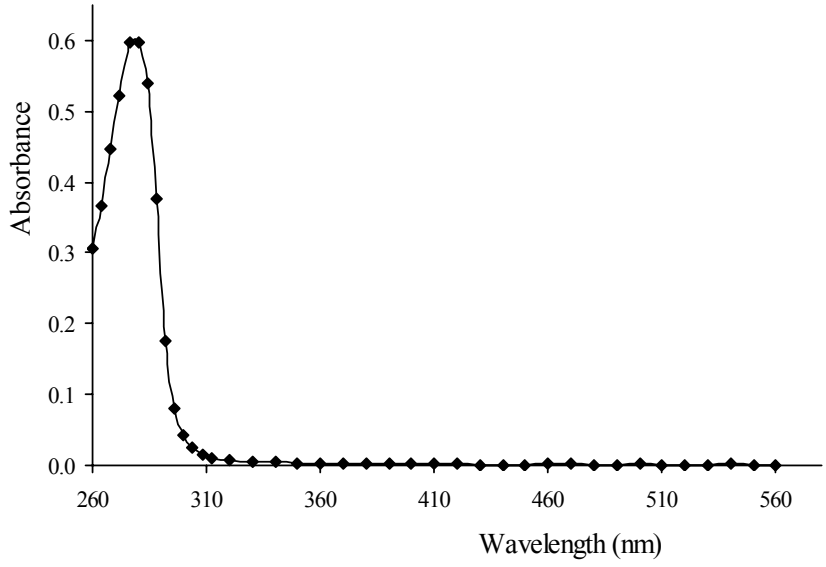

Figure S2. Analysis of purified p50•p66$_N$ sample by UV-visible spectrophotometry.

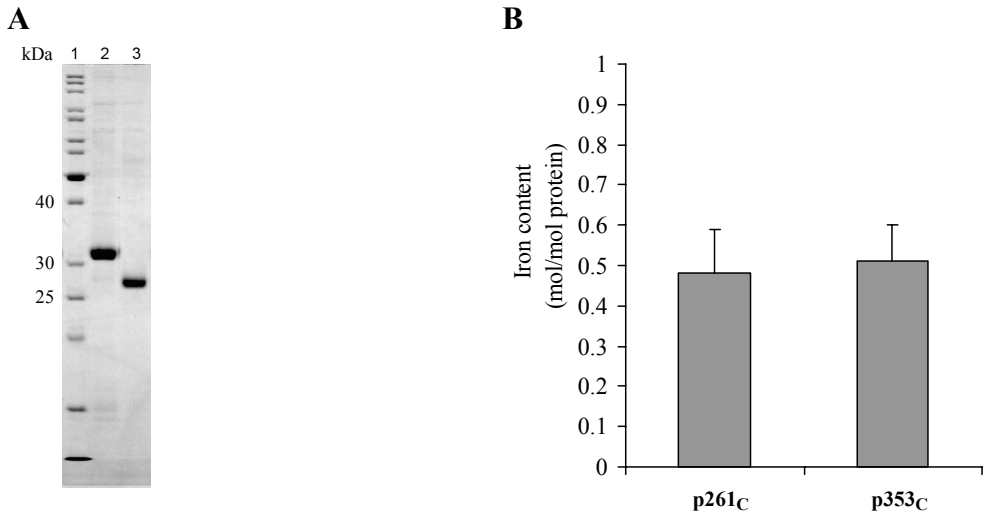

Figure S3. Analysis of purified His_6-SUMO-tagged $p261_C$ and $p353_C$. ***A*,** Purity analysis by electrophoresis in 13% SDS-PAGE with Coomassie Blue staining. Lane 1 – EZ-Run *Rec* protein ladder (Fisher Scientific); lanes 2, 3 – His_6-SUMO-tagged $p261_C$ and $p353_C$, respectively. ***B*,** Iron content in purified His_6-SUMO-CTDs. Error bars, s.d. (n = 3).
Significantly low iron content in the purified His_6-SUMO-CTDs alone probably reflects decreased stability of [4Fe-4S] cluster without a complex with B-subunit.

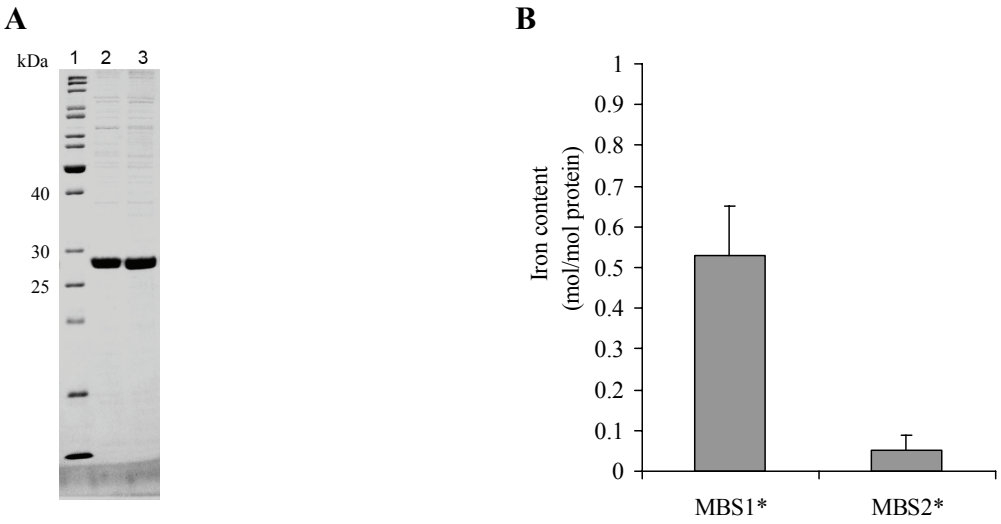

Figure S4. Analysis of purified His_6-SUMO-$p353_C$ mutants. ***A*,** Purity analysis by electrophoresis in 13% SDS-PAGE with Coomassie Blue staining. Lane 1 – EZ-Run *Rec* protein ladder (Fisher Scientific); lanes 2, 3 - His_6-SUMO-$p353_C$ with mutated MBS1 or MBS2, respectively. ***B*,** Iron content in purified His_6-SUMO- $p353_C$ mutants (designated as MBS1* and MBS2*). Error bars, s.d. (n = 3)

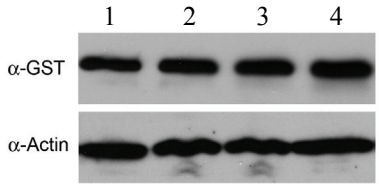

Figure S5. Analysis of Rev3 constructs expression in *S. cerevisiae.* Lane 1 – GST-Rev3 wild-type; lanes 2, 3, 4 – GST-Rev3 with mutated MBS1, MBS2 and both sites, respectively. Yeast extracts were prepared using glass beads in the buffer containing 50 mM Tris-HCl, pH 7.5, 300 mM NaCl, 1 mM EDTA, 10 % sucrose, 10 mM b-ME, 1 mM PMSF, 1X Complete EDTA-free protease inhibitor cocktail (Roche). Lysates were run on 8 % SDS-PAGE for 1 hr at 200 V followed by transfer to Immobilon membrane (Millipore). Mouse anti-GST (Genscript) and goat HRP-conjugated anti-mouse secondary antibodies (Genscript) were used to detect GST-Rev3 fusion protein. Goat anti-human actin antibodies (Santa Cruz Biotechnology, sc-1615; also cross-react with yeast actin), along with the donkey anti-goat HRP-conjugated secondary antibodies (Genscript) were used to detect actin (loading control). Blot was developed using SuperSignal West Femto Chemiluminescent substrate detection kit (Thermo Scientific).

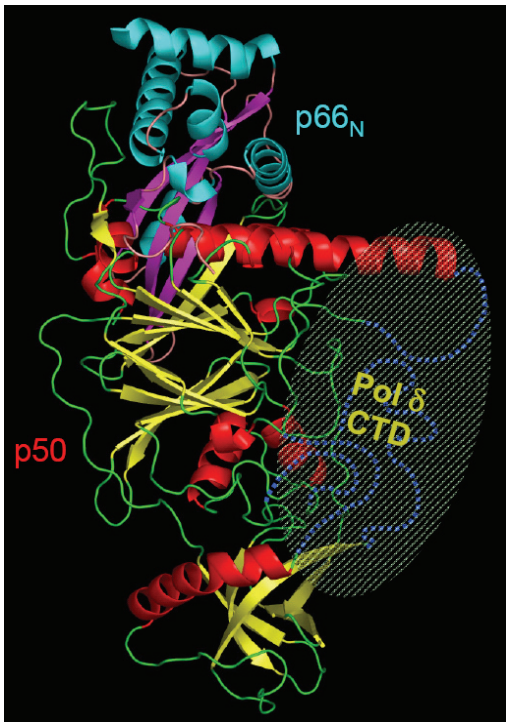

Figure S6. Cartoon representation of p50•$p66_N$ complex (PDB code 3E0J) prepared with PyMol software (Delano Scientific). The secondary structure elements are color-coded as follows: α-helices, β-strands and coils are red, yellow and green, respectively, in p50, and are cyan, magenta and light pink, respectively, in $p66_N$. The modeled disordered regions are shown with dotted lines. The proposed binding site for Pol δ CTD is indicated by transparent oval.

Table S1. Quantification of subunits stoichiometry in Pol δ and Pol ζ p50•66_N•CTD complexes*.

Subunit(s)	Molecular mass, kDa	Relative mass[1]	Relative band intensity[2]	Stoichiometry[3]
Pol δ **p50•66_N•CTD** complex				
p50	52.1	0.65	0.59	0.9
$p66_N$	16.2	0.2	0.21	1.0
$p125_C$	12.5	0.15	0.20	1.3
p50-$p66_N$-$p125_C$	80.8	1	1	
Pol ζ **p50•66_N•CTD** complex				
p50	52.1	0.65	0.58	0.9
$p66_N$	16.2	0.2	0.22	1.0
$p353_C$	11.9	0.15	0.20	1.3
p50-$p66_N$-$p353_C$	80.2	1	1	

*The integrated densities were measured using ImageJ program (v.145s, NIH) and the gel image shown on the Fig.3*A* (two medium lanes with dtUD1-treated samples).
[1] Relative mass - the mass of corresponding subunit divided by the total mass of all subunits in the complex.
[2] Relative band intensity - integrated density of the band of corresponding subunit divided by the total integrated density of all subunits in the complex.
[3] Stoichiometry - relative band intensity divided by the relative mass.

SUPPLEMENTAL REFERENCES

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