Supplementary information

Structure of the human RNA Polymerase I Elongation Complex

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Supplementary Fig. S1 Sample preparation and transcription assay of the human Pol I (a) The purified hPol I consists of all 13 subunits. The complex was subjected to SDS-PAGE followed by Coomassie blue staining. (b) RNA extension and cleavage assay of the purified hPol I shows robust activity. Schematic model of DNA-RNA substrates (only the critical regions shown) used in the assay is shown as in Fig. 1a and Fig. 4a. The translucent nucleotides of the cleavage sequence represent the two nucleotides cleaved in the assay. In the bottom panel, the length of 5'-FAM RNA is labelled. The arrow indicates extension products. The asterisk indicates the RNA products from intrinsic RNA cleavage activity of Pol I. The negative control (NC) of the cleavage assay was performed by adding 5'-FAM-RNA without DNA scaffold to the Pol I at the same condition. (c) The typical negative stain micrograph of apo hPol I shows homogenous monomeric polymerase complex.



Supplementary Fig. S2 Data collection and image processing.

Cryo-EM reconstructions of Pol I EC^{pre} (a), EC^{post} (b), EC^{bt} (c) and EC^{post}-crosslinking (d) accordingly. In each figure, the top panel is the flow-chart of the cryo-EM image processing; the

middle panel shows the representative cryo-EM raw micrograph and 2D classification; the bottom left panel is the FSC curve of the corresponding map; the bottom middle and right panels are the local resolution estimation and orientation of the cryo-EM reconstructions of the corresponding map.



Supplementary Fig. S3 Cryo-EM map and structural model of hPol I EC^{post}.

Overall cryo-EM map of hPol I EC^{post} is shown in the center. The cryo-EM maps of representative regions are shown in mesh and structural models shown in cartoon. Most of the side chains fit in corresponding density, indicating the structure was correctly built.



Supplementary Fig. S4 Structural comparison of human Pol I EC in the three states.

(a) The structural models of human EC^{post} (gray), EC^{pre} (green) and EC^{bt} (orange). (b) Comparison of the DNA-RNA hybrid in the EC^{post} (gray) and EC^{pre} (green). (c) Comparison of the bridge helix and surround regions in the EC^{post} and EC^{bt}. The EC^{post} is colored in gray and EC^{bt} is colored in orange expect for the RPA12 C-ribbon (red). Structural differences are indicated with arrows. (d-e) Close-up views of the C-ribbon of RPA12 in the active site. The cryo-EM map is shown in mesh (d)

and transparent surface (e), respectively. Most of residues are well-fit into the cryo-EM map.



Supplementary Fig. S5 Structural comparison of hPol I with other RNA polymerases.

(a) Structural model of the stalk of yPol I (PDB: 5M3F)¹. A14 is colored in green and other subunits are colored as in hPol I in Fig. 1. (b) The catalytic centers are similar in hPol I EC^{pre} and yPol I EC^{pre} (PDB: 6HKO)². Subunits are colored as in Fig. 1 (c) The FL1, FL2, loopA, and loopB in the active site of hPol I form a narrow tunnel that directs the exit of the nontemplate DNA strand. Pol II (green;

PDB: 5FLM)³ and Pol III (purple; PDB: 7D58)⁴ show distinct conformations of these elements. (d) Comparison of DNA-RNA hybrid in hPol I EC^{bt} in the post-cleavage state and yPol II in the reactivation state (PDB: 3PO3)⁵ with color diagram listed. (e) The conserved residue R56 of RPAC2 in Pol I and Pol III (PDB: 7D58)⁴ makes distinct contact with RPA1/RPC1 in two enzymes.

а		81	
<i>RPA1_HUMAN</i> RPA1_HUMAN RPA1_YEAST	1 1	TT MLISKNMPWRRLOGTSFGMYSAEBLKKLSVKSITNPRYLDSLCNPSANGLYDLALGPADSKEVCSTCVQD MDISKPVGS.EITSVDFGLLTAKEIRNLSAKOITNPTVLDNLCHPVSGCLYDLALG AFLRNLCSTCGLD	clamp core
RPA1_HUMAN RPA1_HUMAN RPA1_YEAST	71 69	FSNCSCHLCHIELPDTVYNPLICHRGSCLNCHMLTCPRAVTELLLCOLRVDEVCALOAVTELER EKFCPCHOCHIELPVCYNPLFRNOLYIYLRASCLFCHHFRLKSVEVHRYACKLRLOYCLDESKLDE	
RPA1 <u>HUMAN</u> RPA1_HUMAN RPA1_YEAST	141 139	00 1LNRFIERPPSASETRESPEOTTEIVONNLGSOCAH HTLGSLNSSMYTDDEATEDNGEGSKQSKDISSTLLNETKSKRSEYWDMATAKALSDGRTTERGSF 05	
<i>RPA1_HUMAN</i> RPA1_HUMAN RPA1_YEAST	181 209	00000000000000000000000000000000000000	clamp head
<i>RPA1_HUMAN</i> RPA1_HUMAN RPA1_YEAST	234 279	20202020 202020 TAGOKDEPLCIEEAOIGKEVLTPTSAREHISALWKNEGFFINVLISGMDDDGME KQAKKLDGSNEASANDEESPDVGRNPTEREKTGSTVILSEEVINILDTVFRREQCVUQVVHSRPN.LSR	
<i>RPA1_HUMAN</i> RPA1_HUMAN RPA1_YEAST	290 348	COOL SRFNPSVFFTDFLVPPSRVRPVSRLGDOMFTNGOTVNHOAVMKDVVLTRKHLALMAQEOKLPEEVATPT KLVKADSFFDVVVVPFRPRLPSKLGBEVHEDSONQLUSKVLTTSLLIRDMNDDLSKLQ	clamp
<i>RPA1_HUMAN</i> RPA1_HUMAN RPA1_YEAST	360 408	all all <th>core</th>	core
<i>RPA1_HUMAN</i> RPA1_HUMAN RPA1_YEAST	427 466	B3 B4 B5 Clocococococococococococococococococococ	dock
<i>RPA1_HUMAN</i> RPA1_HUMAN RPA1_YEAST	497 536	all3 block <	
<i>RPA1_HUMAN</i> RPA1_HUMAN RPA1_YEAST	567 606	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	
<i>RPA1_HUMAN</i> RPA1_HUMAN RPA1_YEAST	637 676	al6 al7 al8 <u>00000 TT 00000 TT</u> SGASMGTRGCFFTRHYMELVYRCTTDKVGRVKLLSPGILKPPPLWTCKOVVSTLLENII AOVWLTSKDSFFTREOXQQYIYGCTRPEDOHTTRSKI VTLPPTIFKPYPLWTCKQIITTVLLNVTPPDPMP	pore
<i>RPA1_HUMAN</i> RPA1_HUMAN RPA1_YEAST	702 746	β11 β12 β13 α19 TT TT 00000000 0 PLNLSGKAKITGKANVKETPRSVPGFNPDSMCGSOVTIREGELLCGVDKAHVGSSAVGLVE.CYFTYGG GINLISKNKIKKNEYMGKGSLENEVLFKDGALLCGILDKSOVGASKYGIVHSLH3VYGP	
<i>RPA1_HUMAN</i> RPA1_HUMAN RPA1_YEAST	772 804	c20 c21 c22 010100100000000000 TTT 000000000000000000000000000000000000	
<i>RPA1_HUMAN</i> RPA1_HUMAN RPA1_YEAST	842 873	C24 2010202020202020202020 VRGKWQDAHLCKDQRDFNMTDLKFKEEVNHYSNETNKACMPFCLHROFPENSLOM/VOSGAKGSTVNTMO PELLKRLQETL <mark>RDN</mark> NKSG <mark>ILDA</mark> VTSSKVNAITSOVVSKCVPDCTMKKFPCNSMOAMALSGAKGSNVNVSQ	funnel
<i>RPA1_HUMAN</i> RPA1_HUMAN RPA1_YEAST	912 943	β14 TT TT 3 β15 2000000000000000000000000000000000000	bridge helix
<i>RPA1_HUMAN</i> RPA1_HUMAN RPA1_YEAST	982 1013	000000000000000000000000000000000000	
<i>RPA1_HUMAN</i> RPA1_HUMAN RPA1_YEAST	1052 1081	a27 a28 a29 a30 a31 10000 100000000000 TT 1000 1000000 1000000 HLHEVUSRADHHFRAIKKWOSKHPNTLLRRGAFLSYSKIQEAVKALKLESENRNGRSPGTQEML NPSALIEHLD VESALKYSKKTLKYRKKHS	foot
<i>RPA1_HUMAN</i> RPA1_HUMAN RPA1_YEAST	1122 1110	043 2020 TT TT TT 2020020202020 RMWYELDEESRRKYQKKAARCPDESLSVMRPDIMFASVSETEETKVDDVSQEWAAQTEKSYEKSILSLDR 	
<i>RPA1_HUMAN</i> RPA1_HUMAN RPA1_YEAST	1192 1166	a33 a34 a35 Jaclolololol TT 10001000000000000000000000000000000	-
<i>RPA1_HUMAN</i> RPA1_HUMAN RPA1_YEAST	1262 1236	β19 → 20020000 200 PMMSVEVLNTKKALKRVKSLKKOLTRVCKGEVEOKIDUOESFCMEEKQNKFQVVOERFOLPHAYYOQ PQMTLCIWN.DVSDEQADTFCKSISKVLMSEVIDKVIVTETGTSNTAGGNAARSYVTHMRTFDNNEYSE	7
<i>RPA1_HUMAN</i> RPA1_HUMAN RPA1_YEAST	1330 1305	CS Q. QUOQUQUQUQUQUQUQUQUQUQUQUQUQUQUQUQUQUQU	jaw
RPA1_HUMAN RPA1_HUMAN RPA1_YEAST	1400 1373	IVDAEAEEGDADASDAKRKEKQEEEVDYESEEEERSGEENDDEDMQERNPHREGARKTQEQDEBVGIG 	
RPA1_HUMAN RPA1_HUMAN RPA1_YEAST	1470 1426	α39 <u>β22</u> <u>β000000000</u> TT TEEDPSIPALLTOPRNPTHSQEPQGPEAMERNVCAVREIHPFIDDVOVDTSESLWCQVTVKDPLMKINFD EQINKSIVEANNMMXVQRD	jaw
RPA1_HUMAN RPA1_HUMAN RPA1_YEAST	1540 1485	α40 β23 β24 η6 α41 0.000.000 + T + T 2.000 0.000 NSSLVVSIAHICAVH MATKGETRCULNETTNIKNEKELVINTEGINLPELFKVAEVEDURELYSNUMAL 0.000	
<i>RPA1_HUMAN</i> RPA1_HUMAN RPA1_YEAST	1610 1551	a42 a43 accontraction accontracti	clamp
RPA1_HUMAN RPA1_HUMAN RPA1_YFAST	1680 1621	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	0016



Supplementary Fig. S6 Sequence alignments of human and yeast Pol I subunits.

(a-c) Sequence alignments of RPA1 (a), RPA2 (b), RPABC2 (c) of hPol I and yPol I homologous

subunits, which are also known as A190, A135 and Rpb6 in yeast, respectively. Structural elements/regions of RPA1 and RPA2 are indicated with dark boxes.

Supplementary Table S1 Statistics of cryo-EM data collection, refinement and validation

statistics.

	EC ^{pre}	EC ^{post}	EC ^{bt}	EC ^{post} -crosslinking
EMDB/PDB	31876/7VBA	31877/7VBB	31878/7VBC	-
Data collection and processing				
Magnification	130,000	130,000	130,000	36,000
Voltage (kV)	300	300	300	200
Total electron exposure (e ⁻ /Å ²)	~50	~50	~50	~50
Exposure rate (e ⁻ /pix/s)	~8	~8	~8	~21
Number of frames per movie	32	32	32	40
Automation software	SerialEM	SerialEM	SerialEM	SerialEM
Defocus range (µm)	-1.5 to -2.5	-1.5 to -2.5	-1.5 to -2.5	-1.5 to -2.5
Pixel size (Å)	1.054	1.054	1.054	1.1
Symmetry imposed	C1	C1	C1	C1
Micrographs (no.)	3,283	2,074	2,854	505
Total of extracted particles (no.)	1,141,229	618,806	676,465	353,482
Total of refined particles (no.)	382,890	282,280	152,653	127,587
Local resolution range (Å)	6.0-2.0	6.0-2.0	6.0-2.0	6.0-2.0
Resolution Masked 0.143 FSC (Å)	2.89	2.81	3.01	3.89
Refinement				
Map sharpening B-factor (Å ²)	100.9	83.6	79.4	-170
Initial model used (PDB code)	5M3F	5M3F	5M3F	
Refinement package	Phenix (real space)	Phenix (real space)	Phenix (real space)	
r.m.s. deviations				
Bond lengths (Å)	0.011	0.013	0.013	
Bond angles (°)	1.181	1.035	1.04	
Validation				
MolProbity score	2.46	2.5	2.51	
All-atom clashscore	17.66	20.11	19.89	
Rotamers outliers (%)	0.62	1.05	0.19	
Cβoutliers (%)	0	0	0	
CaBLAM outliers (%)	10.1	10.47	10.08	
B-factors (min/max/mean)				
Protein	13.95/133.07/53.2	17.91/199.99/72.39	14.30/186.28/79.98	
Ligand	28.47/149.49/84.81	28.78/242.64/147.72	60.15/186.26/136.00	
Overall correlation coefficients				
CC (mask)	0.79	0.75	0.74	
CC (peaks)	0.63	0.57	0.56	
CC (volume)	0.76	0.73	0.72	
Ramachandran plot statistics				

Favored (%)	81.5	82.35	81.4	
Allowed (%)	17.23	16.61	17.72	
Disallowed (%)	1.27	1.05	0.89	

EC ^{post/pre} assembly	
nontemplate DNA	5'-CATTTTGGGCCGCCGGGTTAGGTACTCAGTACTGTCCTCTGGCGAC-3'
template DNA	3'-GTAAAACCCGGCGGCCCAATAACGACTGAGCGACAGGAGACCGCTG-5'
RNA	5'-UGCUGACU-3'
EC ^{bt} assembly	
nontemplate DNA	5'-CATTTTGGGCCGCCGGGTTAGGTACTCAGTACTGTCCTCTGGCGAC-3'
template DNA	3'-GTAAAACCCGGCGGCCCAATAACGACTGTGCGACAGGAGACCGCTG-5'
RNA	5'-UGCUGACU-3'
Extension assay	
nontemplate DNA	5'-GTACTGTCCTCTGGAC-3'
template DNA	3'-ATAACGACTGAGCGACAGGAGACCTG-5'
RNA	5'-FAM-GUGCUGACU-3'
Cleavage assay	
nontemplate DNA	5'-CATTTTGGGCCGCCGGGTTAGGTACTCAGTACTGTCCTCTGGCGAC-3'
template DNA	3'-GTAAAACCCGGCGGCCCAATAACGACTG <u>T</u> GCGACAGGAGACCGCTG-5'

Supplementary Table S2 RNA and DNA oligonucleotides

RPA1	1-5, 282-289, 315-317, 525-532, 1227-1238, 1302-1312, 1363-1495
RPA2	1-4, 1085-1092
RPAC1	1-7, 344-346
RPAC2	1-20, 129-133
RPA43	1-45, 205-338
PAF53	1-5, 116-419
PAF49	1-7, 158-510
RPA12	1-5, 67-79
RPABC1	1-5, 50-55, 211-215
RPABC2	1-50
RPABC3	1-2, 149-150
RPABC4	1-13
RPABC5	65-67

Supplementary Table S3 Disordered regions that were not modeled in the structure.

Supplementary Movie S1 Cryo-EM map and structural model of the Pol I EC^{post}. Color scheme is same as Fig. 1.

Supplementary Movie S2 Structure comparison of hPol I EC and yPol I EC as in Fig. 2.

Supplementary References

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