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Supplemental Information

Structural Basis of RNA Polymerase I

Transcription Initiation

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Table S1. Crystallographic data collection, phasing and refinement statistics for anomalous and native datasets collected from CF crystals. Related to Figure 1

	Native 1	Native 2 (PDB 5N5X)	SeMet 1
Data collection	PX1 Pilatus M6	PX1 EIGER 16M	PX3 Pilatus 2M-F
Space group	P65	P1	P65
Cell dimensions			
a, b, c (Å)	109.09, 109.09, 383.80	109.07 109.14 385.64	108.70, 108.70, 383.40
α, β, γ (°)	90.00, 90.00, 120.00	90.02 90.01 59.98	90.00, 90.00, 120.00
			<i>Peak</i>
Wavelength (Å)	1.00000	0.99998	0.97941
Resolution (Å)	50.0 - 3.2	60.0 - 3.2	50.0 - 4.0
R_{merge} ^a (%)	9.1 (282.9) ^a	9.2 (235.1)	34.3 (227.3)
$I/\sigma(I)$	22.14 (1.38)	14.83 (1.12)	13.50 (2.08)
$CC_{1/2}$	100 (54.0)	99.9 (41.7)	99.9 (73.9)
Completeness (%)	100 (99.9)	98.9 (99.4)	99.9 (100.0)
Redundancy	11.6 (11.8)	8.3 (8.2)	42.7 (42.3)
Refinement			
Resolution (Å)		54.57 - 3.20	
No. reflections		251,065	
$R_{\text{work}} / R_{\text{free}}$ (%)		26.10 / 28.84	
No. atoms		62,886	
Protein		62,730	
Sulfate		150	
Magnesium		6	
B factors (Å ²)		146.98	
Protein		146.92	
Ligand/ion		173.44	
r.m.s deviations			
Bond lengths (Å)		0.008	
Bond angles (°)		1.173	
Ramachandran			
Preferred/allowed/disallowed (%)		84.67/12.98/2.34	

^a Values in parentheses are for highest-resolution shell.

Table S2. Crystallographic data collection and phasing statistics for anomalous datasets of native and methionine mutant CF crystals. Related to Figure 1

	Sulfur (native crystal)	Rrn11_L430M and Rrn7_I408M	Rrn6_L25M and Rrn7_F438M	Rrn11_W84M	Rrn11_L73M and Rrn7_V212M
Data collection	PX1 EIGER 16M	PX1 EIGER 16M	PX1 EIGER 16M	PX1 EIGER 16M	PX1 EIGER 16M
Space group	P65	P65	P65	P65	P65
Cell dimensions					
a, b, c (Å)	108.65, 108.65, 384.60	108.25, 108.25, 381.60	109.05, 109.05, 384.20	109.25, 109.25, 384.60	109.20, 109.20, 384.80
α, β, γ (°)	90.00, 90.00, 120.00	90.00, 90.00, 120.00	90.00, 90.00, 120.00	90.00, 90.00, 120.00	90.00, 90.00, 120.00
		<i>Peak</i>	<i>Peak</i>	<i>Peak</i>	<i>Peak</i>
Wavelength (Å)	2.06640	0.97848	0.97865	0.97986	0.97849
Resolution (Å)	40.0 - 3.4	35 - 4.0	35 - 4.0	35 - 4.5	35 - 4.2
R_{merge} ^a (%)	53.5 (194.1) ^a	46.5 (229.1)	34.9 (223.9)	34.4 (128.3)	37.4 (133.8)
$I/\sigma(I)$	29.64 (2.38)	12.40 (2.02)	12.23 (2.05)	4.30 (1.33)	14.61 (2.25)
$CC_{1/2}$	99.8 (37.2)	99.3 (74.1)	99.8 (78.4)	98.4 (48.7)	99.5 (47.1)
Completeness (%)	99.3 (92.6)	99.9 (100.0)	99.8 (100.0)	99.8 (100.0)	99.6 (97.6)
Redundancy	385.2 (81.5)	36.1 (24.4)	39.5 (30.1)	6.0 (6.1)	51.5 (19.5)

^a Values in parentheses are for highest-resolution shell.

Table S3. Pol I cleft expansion and contraction. Related to Figure 7

	Downstream ^a [Å]	Upstream ^b [Å]	A190 ₄₁₄ relative to 4C2M ^c [Å]
Dimer (4C2M)	42	41	-
Free Monomer (5M3M)	41	39	6
Pol I - Rrn3 (5G5L)	41	36	6
Pol I-Rrn3-CF (5N5Y)	39	36	7
ITC (5N61)	35	29	15
EC (5M3F)	34	28	17

^a Structures were superimposed on A135 subunits. Distances measured between C_α atoms of residues G231 and K1331 in the largest Pol I subunit A190.

^b Distances measured between C_α atoms of residues E414 in subunit A190 and K434 in subunit A135.

^c Displacement of the C_α atom of the A190 clamp core residue E414 towards the protrusion in comparison to the crystal structure 4C2M.

Table S4. dsDNA scaffold sequences used in basal initiation assays. Related to STAR Methods

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides (continued from KEY RESOURCES TABLE)		
Initiation assay template randomized -1 to -10 (dsDNA) GAGTACAAGTGTGAGGAAAAGTAGTTGGGTCTAGC AGTATGCGAAAGCAGTTGAAGACAAGT	This work	NA
Initiation assay template randomized -11 to -20 (dsDNA) GAGTACAAGTGTGAGGAATCCGATGTAGGAGGTAC TTCATGCGAAAGCAGTTGAAGACAAGT	This work	NA
Initiation assay template randomized -21 to -30 (dsDNA) GAGTACAAAGGCCATTGGAAGTAGTTGGGAGGTAC TTCATGCGAAAGCAGTTGAAGACAAGT	This work	NA
Initiation assay template randomized -31 to -38 (dsDNA) GCTACAGAGTGTGAGGAAAAGTAGTTGGGAGGTAC TTCATGCGAAAGCAGTTGAAGACAAGT	This work	NA
Initiation assay template +1ATG to +1GTA (dsDNA) GAGTACAAGTGTGAGGAAAAGTAGTTGGGAGGTAC TTCGTACGAAAGCAGTTGAAGACAAGT	This work	NA
Initiation assay template deleted -1 to -10 (dsDNA) TTTAGTCATGGAGTACAAGTGTGAGGAAAAGTAGTT GGATGCGAAAGCAGTTGAAGACAAGT	This work	NA
Initiation assay template deleted -11 to -20 (dsDNA) TTTAGTCATGGAGTACAAGTGTGAGGAAGAGGTACT TCATGCGAAAGCAGTTGAAGACAAGT	This work	NA
Initiation assay template deleted -21 to -30 (dsDNA) TTTAGTCATGGAGTACAAAAGTAGTTGGGAGGTACT TCATGCGAAAGCAGTTGAAGACAAGT	This work	NA
Initiation assay template deleted -31 to -38 (dsDNA) TTTAGTCAGTGTGAGGAAAAGTAGTTGGGAGGTACT TCATGCGAAAGCAGTTGAAGACAAGT	This work	NA
Initiation assay template deleted +1ATG (dsDNA) GAGTACAAGTGTGAGGAAAAGTAGTTGGGAGGTAC TTCCGAAAGCAGTTGAAGACAAGT	This work	NA