

Supplemental Information

Structural Basis of Poxvirus Transcription:

Vaccinia RNA Polymerase Complexes

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SUPPLEMENTARY TABLES

Table S1: Subunit composition of vRNAP. Related to Figure 1.

vRNAP (kDa)	<i>S.cerevisiae</i> RNA Polymerase II homolog (kDa)	Sequence identity (%)*
Rpo147 (146.9)	Rpb1 (191.6)	17.5
Rpo132 (133.4)	Rpb2 (138.8)	17.1
Rpo35 (35.4)	Rpb3 (35.3) / Rpb11 (13.6)	5.7 / 2.8
Rpo30 (29.8)	TFIIS	11.4
Rpo22 (21.3)	Rpb5 (25.1)	11.5
Rpo19 (19.0)	Rpb6 (17.9)	19.6
Rpo18 (17.9)	Rpb7 (19.1)	12.7
Rpo7 (7.3)	Rpb10 (8.3)	22.9
Rap94 (93.6)	-	-

* Sequence identity was calculated using structure-based sequence alignments and using Ident and Sim available at https://www.bioinformatics.org/sms2/ident_sim.html.

Table S3: Identification of the tRNA species bound to complete vRNAP by RNAseq analysis. Related to Figure 1.

	number of reads	%
tRNA ^{Gln} (TTG)	633503	98.2
tRNA ^{Gln} (CTG)	7057	1.1
tRNA ^{Arg} (ACG)	3337	0.5
tRNA ^{Arg} (TCG)	1745	0.2

Table S4: Cryo-EM data collection, refinement and validation statistics. Related to Figure 2 and Figure 4.

	Core vRNAP (EMD-4888) (PDB 6RIC)	Complete vRNAP (EMD-4868) (PDB 6RFL)
Data collection and processing		
Magnification	165,000	75,000
Voltage (kV)	300	300
Electron exposure (e ⁻ /Å ²)	55	50
Defocus range (μm)	-0.6 to -2.5	-1.4 to -2.7
Pixel size (Å)	0.81	1.06
Symmetry imposed	C1	C1
Initial particle images (no.)	479,618	858,702
Final particle images (no.)	152,591	618,338
Map resolution (Å)	2.8	2.75
FSC threshold	0.143	0.143
Map resolution range (Å)	2.8 – 4.8	2.6 – 5.0
Refinement		
Model resolution (Å)	2.9	2.9
FSC threshold	0.5	0.5
Map sharpening <i>B</i> factor (Å ²)	-79	-39
Model composition		
Non-hydrogen atoms	29,677	51,788
Protein / RNA residues	3,663 / 0	6,194 / 63
Ligands / Water	4 x Zn, 1 x Mg / -	4 x Zn, 1 x Mg / 61
<i>B</i> factors (Å ²)		
Protein/RNA	52.3 / -	55.3 / 75.5
Ligand / Water	89.4 / -	49.0 / 30.2
R.m.s. deviations		
Bond lengths (Å)	0.006	0.005
Bond angles (°)	0.751	0.756
Validation		
MolProbity score	1.49	2.15
Clashscore	5.10	4.30
Poor rotamers (%)	0.03	4.82
Ramachandran plot		
Favored (%)	96.6	93.7
Allowed (%)	3.5	6.2
Disallowed (%)	0.00	0.05

Table S5: X-Ray data collection, refinement and validation statistics. Related to Figure 4.

	E11-native (PDB 6RFG)	E11-Hg
Data collection		
Space group	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁
Cell dimensions a,b,c (Å)		59.8, 75.5, 138.8
Resolution (Å)	40.0 - 1.90 (2.00 - 1.90)*	40.0 – 2.29 (2.43 – 2.29)* [40.0-6.86]**
Rsym (%)	16.4 (163.5)	6.4 (24.7)
I/σ(I)	11.1 (1.0)	17.6 (5.2)
Completeness (%)	96.6 (87.4)	94.4 (69.3)
Redundancy	6.4 (6.1)	4.8 (3.9)
CC1/2 (%)	99.7 (34.9)	99.8 (92.6)
SigAno		0.99 (0.80) [2.24]
Anomalous correlation (%)		19.0 (1.0) [75.0]
Refinement (Phenix.refine)		
Resolution (Å)	40.0 - 1.90 (2.00 - 1.90)	
No. of reflections	48722 (4328)	
Rwork / Rfree (%)	20.7 / 22.9 (33.6 / 38.1)	
Model composition		
Non-hydrogen atoms	4584	
Protein residues	520	
Water molecules	333	
RMS deviations		
Bond lengths (Å)	0.003	
Bond angles (°)	0.78	
Validation		
All-atom clashscore	2.47	
Rotamer outliers	1.64 %	
C-beta deviations	0	
Ramachandran plot		
Outliers	0.2	
Allowed	2.9 %	
Favored	96.9 %	

* Number in () parentheses for highest resolution shell

** Number in [] parentheses for lowest resolution shell