Expansion of poxvirus RNA polymerase subunits sharing homology with corresponding subunits of RNA polymerase II

Bruce A. Knutson · Steven S. Broyles

Abstract Poxvirus-encoded RNA polymerases were known previously to share extensive sequence homology in their two largest subunits with the corresponding subunits of cellular RNA polymerases and a modest alignment between the smallest poxvirus subunit and RBP10 of RNA polymerase II. The remaining subunits had no apparent cellular homologs. In this study, the HHpred program that combines amino acid sequence alignments with secondary structure predictions was used to search for homologs to the poxvirus RNA polymerase subunits. Significant matches of vaccinia RNA polymerase 22-, 19-, and 18-kDa subunits to RNA polymerase II subunits RPB5, 6, and 7, respectively, were identified. These results strengthen the concept that poxviral RNA polymerases likely evolved from cellular RNA polymerases.

Keywords Vaccinia virus · Poxvirus · RNA polymerase · Transcription

Introduction

Poxviruses are large DNA genome viruses that progress through their replicative cycle exclusively in the cytoplasm of the infected cell (reviewed in [1]). The virus' ability to avoid the cell nucleus is possible in no small part because of the RNA polymerase (Pol) encoded by the virus. This enzyme is responsible for the synthesis of all three classes of viral mRNAs. The Pol of the prototypal poxvirus, vaccinia, has been studied most intensively. The viral Pol requires auxiliary factors to target transcriptional start sites. The virus-encoded early transcription factor targets the early promoter elements [2], and the cellular TATA-binding factor (TBP) targets the upstream promoter elements in intermediate and late promoters [3]. Thus, the vaccinia Pol resembles eukaryotic multiple subunit Pols in its inability to recognize transcriptional start sites on its own.

The vaccinia Pol core enzyme is composed of 8 subunits [4] and is believed to be responsible for the transcription of intermediate and late genes [5]. An additional 94-kDa subunit that is a component of a subset of the total vaccinia Pol population is essential for early gene transcription initiation and termination. It is believed to be a docking platform for early transcription factors [6, 7]. The 147-kDa largest subunit and the second largest 133-kDa subunit of the vaccinia enzyme have extensive sequence homology to corresponding subunits of cellular Pols [8–10]. The two largest subunits comprise most of the active site of the enzyme [11–13]. The high degree of similarity of these subunits to those of Pol II has suggested that poxvirus Pols may have evolved from cellular Pol II [14]. A limited similarity in the smallest 7-kDa subunit of the vaccinia enzyme to that of yeast Pol II RPB10 subunit was noted [15]. Finally, the 30-kDa subunit of the vaccinia Pol was shown to have significant similarity to eukaryotic transcription elongation factor SII [16]. Matches to the remaining four subunits of the vaccinia Pol are not identified by standard BLAST approaches.

B. A. Knutson · S. S. Broyles (✉)
Department of Biochemistry, Purdue University,
West Lafayette, IN 47906, USA
e-mail: broyles@purdue.edu

Present Address:
B. A. Knutson
Fred Hutchinson Cancer Research Center, 1100 Fairview Ave.
N., Mailstop A1-162, Seattle, WA 98109-1024, USA
e-mail: bknutson@fhcrc.org

Methods

Computational approach

Vaccinia virus Pol amino acid sequences were converted to fasta format and analyzed by the HHpred program [17, 18] available at the Max Planck Institute for Developmental Biology at Tuebingen, Germany, whose url is http://www.toolkit.tuebingen.mpg.de/. All searches were conducted with the program's default settings. The program searches the Protein Data Bank for primary sequences and SCOP for secondary structures. Primary sequences were aligned with the CLUSTALX program.

Results

Rpo22

A database search with the vaccinia virus rpo22 sequence using an HHpred approach yielded several significant matches. The highest-ranked hit was Pol subunit RPB5 from the archaea *Methanobacterium thermoautotrophicum*. The second-ranked match was to that of Pol II subunit RPB5 from the yeast *Saccharomyces cerevisae*, and the third-ranked hit was from the same subunit from another archaea, *Methanococcus jannaschii* (Fig. 1). A high-resolution 3-dimensional structure for yeast Pol II is available [13] allowing predicted secondary structures to be tested for accuracy. The secondary structure prediction by the

HHpred program shows a very good correlation with the known structure of yeast RPB5. Alignment of the predicted vaccinia rpo22 secondary structure with that of RPB5 shows many similarities. All the predicted α-helices in rpo22 align with helices in yeast RPB5 except α6 (Fig. 1). Predicted β-folds β5, β7, β8, and β10–12 also align well with β-folds in RPB5. The HHpred program ranked the match between rpo22 and RPB5 as having a probability of 68% and a modest E-value of 1.4 (Table 1). The relative identity between the two proteins is 21% when conservative amino acid replacements are included into the calculation. Of the 185 amino acids in rpo22, there are 37 residues identical with the human and yeast RPB5. This subunit forms part of the "jaw" structure in Pol II [13]. While the archeal subunits are ranked highly against rpo22, they lack about two-thirds of the N-terminal primary sequence of the vaccinia and Pol II subunits (Fig. 1). This suggests that rpo22 would seem more similar to eukaryotic RPB5.

Rpo19

Searching vaccinia virus rpo19 against the database with HHpred revealed a first-ranked match with the Pol II subunit RPB6. There was overall a very good correlation between predicted rpo19 secondary structure and that of RPB6 (Fig. 2). The predicted secondary structures in the N-termini of the two proteins have few α-helices or β-folds, but the structure of this part of the RPB6 protein is
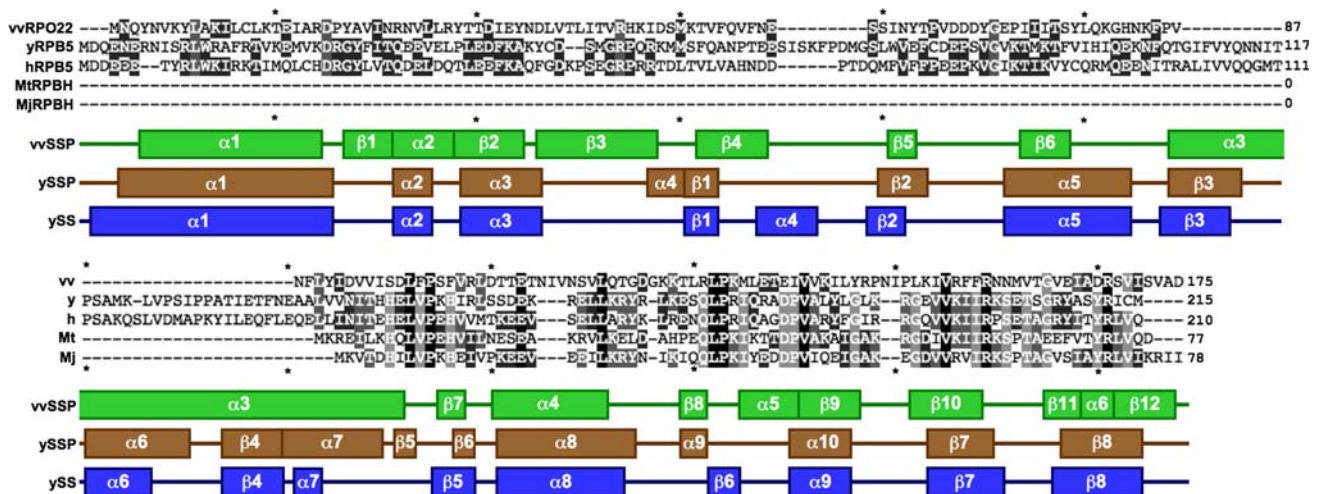

Fig. 1 Alignment of vaccinia virus RNA polymerase subunit rpo22 with yeast and human RPB5. The sequences of vaccinia rpo22 (vvRPO22), yeast (y), human (h), *Methanobacterium thermoautotrophicum* (Mt RPBH), and *Methanococcus jannaschii* (MjRPBH) RPB5 were aligned by the CLUSTALX program. Amino acids conserved between two species are boxed in gray, those conserved for all three are boxed in black. Asterisks demark 20 residue intervals. Below the sequence alignments are the predicted vaccinia rpo22 secondary structure (vvSSP, green), predicted yeast (ySSP, brown), and actual yeast secondary structure (ySS). α-helices and β-fold are indicated as boxes. Coiled regions are indicated by lines

Table 1 HHpred search results for vaccinia virus RNA polymerase subunits

Vaccinia virus RNA polymerase subunit	RNA polymerase II match	Probability (%)[a]	E-value[b]	Identity (%)[c]
Rpo147[d]	RPB1	100	0	20
Rpo132[d]	RPB2	100	0	22
Rpo30[d]	SII	100	4.1e–44	16
Rpo22	RPB5	65	1.4	21
Rpo19	RPB6	90	4.1e–06	20
Rpo18	RPB7	97	0.012	12
Rpo7	RPB10	84	0.21	27

[a] Calculated as the probability that two pairwise comparisons will emit the same sequence

[b] Calculated as the average expected number of non-homologous proteins with a score higher than the one obtained for the database match

[c] Identical and conservative amino differences are included

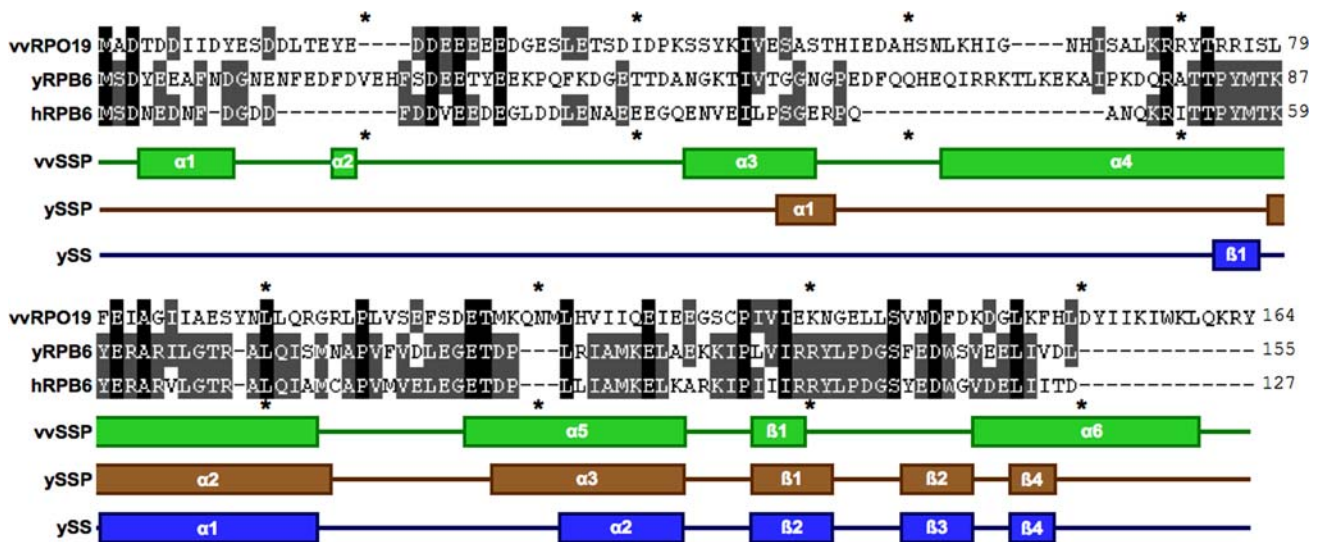[d] Homology previously reported

Fig. 2 Alignment of vaccinia virus RNA polymerase subunit rpo19 with yeast and human RPB6. Sequence alignments and secondary structures are as described in Fig. 1

apparently disordered [13]. This is also predicted to be the case for the N-terminus of rpo19 (data not shown). The predicted vaccinia α-helices 3, 4, and 5 overlap helices in yeast RPB6 as does a β-fold at β1. The HHpred program assigned this alignment a probability of 91% and a very good E-value of 4.0e-06 (Table 1). The amino acid identity was 20% including matches throughout the length of both polypeptides.

Rpo18

A search of vaccinia rpo18 with HHpred identified Pol II subunit RPB7 as the highest-rated match. The predicted secondary structure of rpo18 is remarkably similar to that of RPB7 (Fig. 3). A predicted α-helix near the N-terminus of rpo18 aligns well with a helix in RPB7, and all the 14

predicted β-folds except β2 and β4 align with β-folds of RPB7. The α-helix wrapped by a four-stranded antiparallel β-sheet is a highly conserved structure in the RPB7 family [19]. The sequence identity between the two proteins is low at 12% (Table 1). Nonetheless, the HHpred program rates the match at 97% probability and an E-value of 0.12.

Rpo7

The HHpred program identified a match of vaccinia rpo7 with Pol II subunit RPB10 as the highest-ranking hit. This finding is in agreement with a previous report [15]. There is a 27% sequence identity between the two proteins, higher than that reported previously apparently due to an incorrect yeast sequence. Significantly the second cysteine pair of the zinc finger domain previously was not identified. The
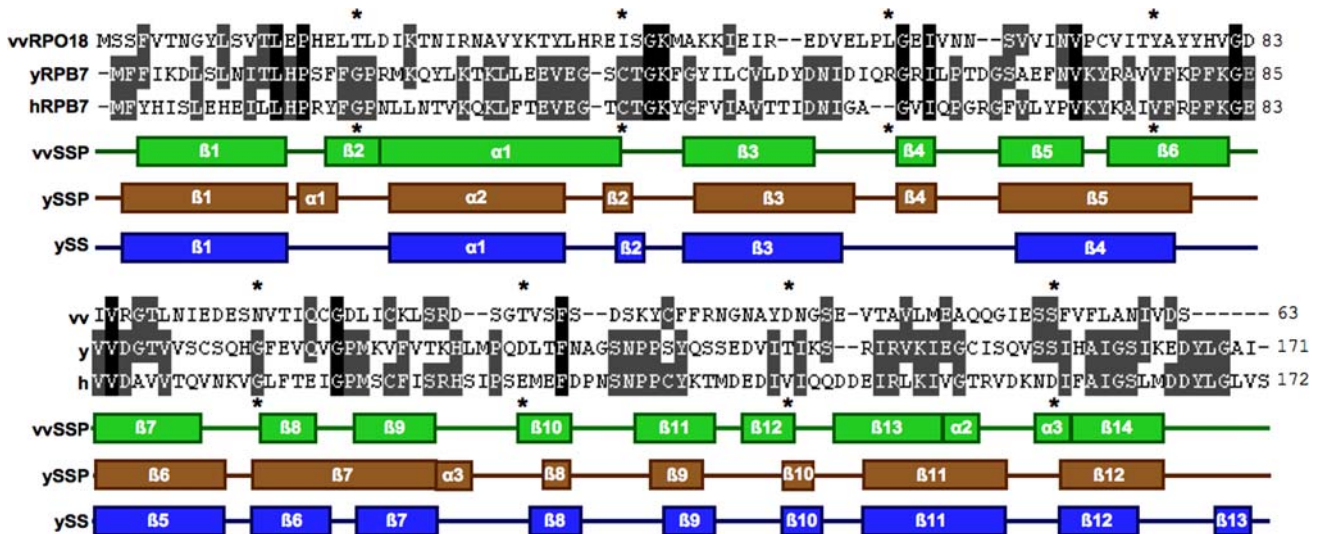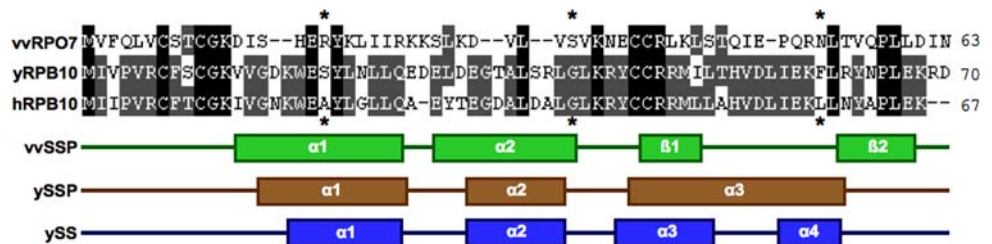
Fig. 3 Alignment of vaccinia virus RNA polymerase subunit rpo18 with yeast and human RPB7. Sequence alignments and secondary structures are as described in Fig. 1

Fig. 4 Alignment of vaccinia virus RNA polymerase subunit rpo7 with yeast and human RPB10. Sequence alignments and secondary structures are as described in Fig. 1

secondary structure prediction identified matches with α-helices 1 and 2 inside the two zinc finger cysteine pairs, but not outside these sequences (Fig 4). The program assigned the match an 84% probability and an E-value of 0.21 (Table 1).

Discussion

The results presented here underscore the value of combining secondary structure information with more traditional sequence alignments in identifying distantly related members of protein families. Three vaccinia virus Pol subunits with no previously identified cellular homologs are shown here to be highly related to Pol II subunits. Vaccinia rpo22, 19, and 18 are very similar to the RPB 5, 6, and 7 subunits of Pol II. All three have reasonably high probabilities in their matches and have respectable levels of amino acid identities in comparison with the more related rpo147 and 132 subunits (Table 1). With the exception of rpo22, all have E-values less than one. It is interesting to note that RPB5, 6, and 10 are shared among the three eukaryotic Pols [20]. The only vaccinia virus core Pol subunit without an apparent match is rpo35. It is noted that

the vaccinia rpo94 that functions specifically for early transcription also appears to have no apparent cellular homolog by the searches employed here. This is perhaps not surprising given that the early transcription factors also have no cellular homologs. HHpred searches with Pol subunits from very divergent poxviruses such as canarypox and entomopoxviruses also identify cellular Pol subunit homologs (not shown). One exception is entomopox that lacks an rpo22 homolog.

It is rather surprising that the vaccinia Pol has no apparent homolog to Pol II RPB3. The latter polypeptide appears to have an integral role in Pol II assembly, linking RPB8, 10, 11, and 12 to the catalytic subunits RPB1 and 2 [11]. Perhaps rpo35 fulfills this function for the vaccinia Pol but has diverged in amino acid sequence too far for recognition. RPB3 has two distinct conserved motifs that should be present in rpo35 if it is a distant homolog: a metal binding site, and a leucine zipper motif near the middle and C-terminus, respectively. Neither of these motifs are present in rpo35. Alternatively, because the vaccinia Pol lacks homologs to RPB8, 11, and 12, an RPB3 equivalent may be unnecessary for vaccinia Pol assembly. RPB3 is also believed to be targeted by multiple eukaryotic transcription factors (Ref. 21 and references therein).
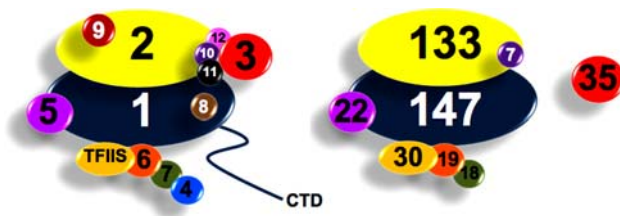
Fig. 5 Image depicting the intersubunit interactions of the subunits of Pol II (on left) and the predicted structure of vaccinia virus Pol (on right). The image of Pol II was adapted from Ref. 13

Vaccinia virus transcriptional promoters are believed to be structured as minimal "core" elements not involving the function of upstream activating sequences, and therefore would not require a "targeting" subunit in its Pol.

Vaccinia Pol also lacks an equivalent to RPB4. This Pol II subunit can form a heterodimer with RPB7 and is believed to stabilize the interaction of RPB7 with the core enzyme [22]. When associated with the RBP4/RPB7 heterodimer, the Pol is in a conformation in which the "clamp" is closed, suggesting that RPB7 blocks the initiation of transcription [22]. The vaccinia RPB7 homolog rpo18 apparently does not require an RPB4 equivalent to stably associate with the Pol enzyme. It is interesting to note that yeast RPB4 is required for recruitment of the RPB1 C-terminal domain (CTD) phosphatase Fcp1 that functions in recycling Pol II through transcriptional initiation [23]. The vaccinia Pol rpo147 subunit lacks a CTD [8]. The RPB4/RPB7 heterodimer has been shown to bind RNA *in vitro*, and the RNA-binding activity is inherent to the RPB7 subunit [19]; however, the significance of this activity to the transcriptional process is unclear. It is also interesting to note that yeast do not require functional RPB4 or RPB7 under normal growth conditions. Mutations in the vaccinia gene encoding rpo18 confer conditional lethality [24], indicating that this Pol subunit is essential to the virus.

The findings presented here suggest that poxvirus Pols have a structural architecture that closely resembles that of cellular Pols. It is reasonable to expect the two largest subunits to have the "crab claw" appearance characteristic of bacterial and eukaryotic Pols (Fig. 5). The subunits rpo18, 19, 22, and 30 are predicted to be associated with

the rpo147 subunit, while rpo7 would be bound to rpo133. The location of rpo35 in this model remains unknown. The proposed model for vaccinia Pol will provide the basis for a framework for understanding the structure and function of poxvirus Pols.

References

1. B. Moss, in *Fields Virology*, ed. by D.M. Knipe, P.M. Howley (Lippincott, Williams & Wilkins, Philadelphia, 2001), pp. 2849–2884
2. S.S. Broyles, J. Gen. Virol. **84**, 2293–2303 (2003)
3. B.A. Knutson, X. Liu, J. Oh, S.S. Broyles, J. Virol. **80**, 6784–6793 (2006)
4. B. Moss, in *Transcription mechanisms and regulation*, vol. 3, ed. by R.C. Conaway, J.W. Conaway, (Raven Press, New York, 1994), pp. 185–205
5. C.F. Wright, A.M. Coroneos, J. Virol. **69**, 2602–2604 (1995)
6. B.Y. Ahn, P.D. Gershon, B. Moss, J. Biol. Chem. **269**, 7552–7557 (1994)
7. R.C. Condit, E.G. Niles, Biochim. Biophys. Acta. **1577**, 325–336 (2002)
8. S.S. Broyles, B. Moss, Proc. Nat. Acad. of Sci. USA **83**, 3141–3145 (1986)
9. D.D. Patel, D.J. Pickup, J. Virol. **63**, 1076–1086 (1989)
10. B.Y. Amegadzie, M. Holmes, N.B. Cole, E.V. Jones, P.L. Earl, B. Moss, Virology **180**, 88–98 (1991)
11. A.L. Gnatt, P. Cramer, J. Fu, D.A. Bushnell, R.D. Kornberg, Science **292**, 1876–1882 (2001)
12. G. Zhang, E.A. Campbell, L. Minakhin, C. Richter, K. Severinov, S.A. Darst, Cell **98**, 811–824 (1999)
13. P. Cramer, D.A. Bushnell, R.D. Kornberg, Science **292**, 1863–1876 (2001)
14. K.C. Sonntag, G. Darai, Virus Genes **11**, 271–284 (1995)
15. B.Y. Amegadzie, B.Y. Ahn, B. Moss, J. Virol. **66**, 3003–3010 (1992)
16. B.Y. Ahn, P.D. Gershon, E.V. Jones, B. Moss, Mol. Cell. Biol. **10**, 5433–5441 (1990)
17. J. Söding, Bioinformatics **10**, 5433–5441 (1990)
18. J. Soding A. Biegert A.N. Lupas, Nucleic Acids Res. **10**, W244–W248 (1990)
19. H. Meka, F. Werner, S.C. Cordell, S. Onesti, P. Brick, Nucleic Acids Res. **33**, 6435–6444 (2005)
20. N.A. Woychik, M. Hampsey, Cell **108**, 453–463 (2002)
21. M. Oufattole, S.W.J. Lin, B. Liu, D. Mascarenhas, P. Cohen, B.D. Rodgers, Endocrinol. **147**, 2138–2146 (2006)
22. K.-J. Armache, H. Kettenberger, P. Cramer, Proc. Nat. Acad. Sci. USA **100**, 6964–6968 (2003)
23. M. Kimura, H. Suzuki, A. Ishihama, Mol. Cell. Biol. **22**, 1577–1588 (2002)
24. J. Seto, L.M. Celenza, R.C. Condit, E.G. Niles, Virology. **160**, 110–119 (1987)