

Review

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Vaccinia virus transcription

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Vaccinia virus replication takes place in the cytoplasm of the host cell. The nearly 200 kbp genome owes part of its complexity to encoding most of the proteins involved in genome and mRNA synthesis. The multisubunit vaccinia virus RNA polymerase requires a separate set of virus-encoded proteins for the transcription of the early, intermediate and late classes of genes. Cell fractionation studies have provided evidence for a role for host cell proteins in the initiation and termination of vaccinia virus intermediate and late gene transcription. Vaccinia virus resembles nuclear DNA viruses in the integration of viral and host proteins for viral mRNA synthesis, yet is markedly less reliant on host proteins than its nuclear counterparts.

INTRODUCTION

Poxviruses are a family of large DNA genome viruses pathogenic for many species of mammals, birds and insects. Their genomes are dsDNA molecules, ranging from 130 kbp (kbp) in parapoxviruses to about 300 kbp in avipoxviruses, with hairpin termini (reviewed by Moss, 2001). A feature that distinguishes poxviruses from other classes of DNA viruses is that the virus remains in the cell cytoplasm for the duration of the infectious cycle from the time the virus enters the cell until the progeny viruses exit through the plasma membrane (Minnigan & Moyer, 1985). This scenario has fostered the notion that poxviruses have evolved to a high level of independence from the host cell, especially for processes involved in DNA replication and mRNA synthesis that otherwise would be expected to occur within the nucleus. It is becoming increasingly clear, however, that poxviruses borrow several host proteins to synthesize mRNA. Poxviruses distinguish themselves from nuclear DNA viruses in the relative proportion of transcriptional proteins encoded by the virus relative to those provided by the host cell.

Temporal regulation of gene expression

The majority of information on the regulation of gene expression in poxviruses has come from studies on the laboratory prototype poxvirus, vaccinia virus. There is ample reason to regard vaccinia virus as a model for transcriptional regulation in all poxviruses. Transcriptional mechanisms appear to be conserved across the entire family of poxviruses. The RNA polymerase and transcription factor genes have been found in all poxviruses for which genome sequences are available, which includes representatives from all poxvirus genera except entomopoxvirus C. Transcription factors and promoter function appear to be conserved, since

promoters from one type of poxvirus are functional in a cell infected with a different poxvirus (Kumar & Boyle, 1990; Tripathy & Wittek, 1990). Like most other classes of virus, poxviruses coordinate the processes of genome replication and virion assembly through regulation of the timing of expression of individual genes. Proteins participating in DNA replication (Jones & Moss, 1984; Lee-Chen & Niles, 1988; Smith *et al.*, 1989a), nucleotide biosynthesis (Hruby & Ball, 1982; Smith *et al.*, 1989b) and intermediate gene transcription (Jones *et al.*, 1987; Lee-Chen & Niles, 1988; Ahn *et al.*, 1990; Broyles & Pennington, 1990; Sanz & Moss, 1999) are synthesized as early class genes, and those participating in virion morphogenesis and assembly tend to be expressed as post-replicative intermediate and late class gene products (Rosel & Moss, 1985). Apparently, it is advantageous to accumulate many copies of the genome before any virus assembly is to proceed. Proteins involved in the evasion of host defences tend also to be early class gene products (Kotwal *et al.*, 1989; Moore & Smith, 1992; Ng *et al.*, 2001). The control of gene expression is exerted at the level of transcription initiation and occurs through a cascade mechanism. The transcription factors required for intermediate genes are expressed as early proteins, factors required for late genes are intermediate gene products and those required for transcription of early genes are late gene products packaged inside progeny virions for use in the next cycle of infection. One early vaccinia virus promoter was shown to reactivate late in the infectious cycle (Garces *et al.*, 1993). The significance of reactivation of early promoters is unclear. It should be noted that a number of vaccinia virus genes have been described as being continuously transcribed throughout the infectious cycle. Usually this is accomplished by a tandem arrangement of early and intermediate or late promoters preceding the open reading frame (for examples, see Wittek *et al.*, 1980; Broyles & Moss, 1986; Ahn *et al.*, 1990; Broyles & Pennington, 1990).

Virtually all viruses, whether containing DNA or RNA

genomes, couple the switch from early to late gene expression to genome replication, and vaccinia virus is no exception. Inhibition of DNA synthesis, either with chemical inhibitors or with conditional lethal mutations that block DNA replication, results in the persistence of early gene transcription and the inhibition of intermediate (Vos & Stunnenberg, 1988) and subsequent late gene transcription. With no DNA synthesis, no transcriptional switch occurs. Curiously, intermediate promoters when transfected into a virus-infected cell override the block by inhibition of DNA synthesis and actually continue to be transcribed at levels higher than when DNA synthesis is allowed to proceed normally. The continued transcription of transfected intermediate genes in the absence of DNA synthesis presumably occurs because the onset of late transcription does not occur and this must somehow limit intermediate transcription under normal conditions. The resistance of transfected intermediate promoters to the inhibition of DNA synthesis has been attributed to a requirement for a 'naked' DNA template for intermediate transcription (Keck *et al.*, 1990). This concept posits that vaccinia virus DNA is relatively free of proteins after the onset of DNA replication; however, we have little information on the proteins that associate with viral DNA in the cell cytoplasm.

Vaccinia virus RNA polymerase

All three classes of vaccinia virus genes are transcribed by the virus-encoded RNA polymerase. This enzyme is remarkably complex, being composed of nine subunits totalling more than 500 kDa in mass (Table 1) (Moss, 1994). The 147 and 136 kDa subunits show a high degree of amino acid similarity to the two largest subunits of eukaryotic and prokaryotic cellular RNA polymerases (Broyles & Moss, 1986; Patel & Pickup, 1989). In the bacterial RNA polymerase and RNA polymerase II from yeast, these two subunits come together to form a crab claw-shaped structure with a cleft that is the site of template interaction and the active site for phosphodiester bond formation (Davis *et al.*, 2002; Murakami *et al.*, 2002). The other subunits interact with the opposite face of the protein, distant from the catalytic site of the enzyme and, thus, are proposed to interact with transcription factors. Other than a modest similarity between the smallest vaccinia virus RNA polymerase subunit (7 kDa) and the smallest subunit of yeast RNA polymerase II (RBP10) (Amegadzie *et al.*, 1992a), the smaller subunits of vaccinia virus RNA polymerase have no significant resemblance to the smaller subunits of cellular RNA polymerases, possibly owing to the lack of sequence similarity to any vaccinia virus transcription factors to known cellular transcription factors.

Early gene transcription

Vaccinia virus early class mRNA appears within minutes after virus entry into the cell. The virion core particle apparently retains much of its structural integrity after cell entry. Viral mRNA is synthesized within the confines of the core particle and is extruded through pores in its surface

(Kates & McAuslan, 1967; Munyon *et al.*, 1967). This is possible because all the enzymes and other proteins required to synthesize mature mRNA are packaged within the virion core along with the DNA genome. These proteins include RNA modification enzymes such as the mRNA capping enzyme, poly(A) polymerase and a 2'-O-methyltransferase in addition to the RNA synthesis machinery.

Approximately half of the vaccinia virus genes belong to the early class (Oda & Joklik, 1967). A single early promoter, that of the 7.5K gene, has been characterized in detail (Davison & Moss, 1989b). Early promoters can be studied *in vivo* only in the context of being resident in the viral genome. Transfected early promoter/reporter gene constructs are not functional (unpublished observations), presumably because they cannot access the interior of the core particle where the salient proteins reside. Analysis of the 7.5K promoter identified a single essential element upstream of the transcription start site spanning nt -12 to -29. Inspection of a number of early promoters reveals that each has a nearly universal G residue at -21 or -22 that is flanked by a sequence that is variable but highly A-T rich (Fig. 1) (Davison & Moss, 1989b).

The initiation of early mRNA synthesis is accomplished by a remarkably simple set of proteins. Highly efficient transcription reactions can be reconstituted *in vitro* on the early gene template using only the viral RNA polymerase and a single transcription factor, the vaccinia virus early transcription factor (ETF) (Broyles *et al.*, 1988). ETF is a heterodimer of the viral D6R and A7L gene products (Broyles & Fesler, 1990; Gershon & Moss, 1990) and is the only known poxvirus-encoded promoter-binding protein. It interacts with two regions of early promoters: nt -12 to -29, relative to the transcription start site, and nt +7 to +10, downstream of the transcription start site (Broyles *et al.*, 1991; Cassetti & Moss, 1996). Thus, the factor contacts the promoter on both sides flanking the transcription start site, apparently without blocking the template at the site of initiation. The -12 to -29 contacts correspond to the early promoter element identified by Davison & Moss (1989b), thus accounting for the promoter sequence requirements. Methylation interference experiments indicate that ETF contacts the invariant G residue within this sequence as well as A residues in the minor groove of the DNA helix (Broyles *et al.*, 1991; unpublished results). Minor groove contacts explain the variation in sequence tolerated by ETF because the chemical groups presented in the minor groove of an A-T base pair are very similar to that of a T-A base pair. The downstream contacts made by ETF are not sequence specific, appearing to be 'mooring' contacts that stabilize the protein-DNA complex. The ETF-promoter complex recruits the RNA polymerase to the site of initiation (Li & Broyles, 1993) and RNA synthesis presumably resumes thereafter.

The initiation of early mRNA synthesis requires ATP as an energy source that is distinct from the adenosine nucleotide incorporated into RNA. ATP analogues with a

Table 1. Proteins participating in vaccinia virus transcription

Protein	Encoding gene	Reference
RNA polymerases		
147 kDa	J6R	Broyles & Moss (1986)
133 kDa	A24R	Patel & Pickup (1989) Amegadzie <i>et al.</i> (1991b)
94 kDa	H4R	Ahn & Moss (1992)
35 kDa	A29L	Amegadzie <i>et al.</i> (1991a)
30 kDa	E4L	Ahn <i>et al.</i> (1990) Broyles & Pennington (1990)
22 kDa	J4R	Broyles & Moss (1986)
19 kDa	A5R	Ahn <i>et al.</i> (1992)
18 kDa	D7R	Ahn <i>et al.</i> (1990b) Quick & Broyles (1990)
7 kDa	G5·5R	Amegadzie <i>et al.</i> (1992a)
Early transcription factors		
82 kDa	A7L	Gershon & Moss (1990)
70 kDa	D6R	Broyles & Fesler (1990) Gershon & Moss (1990)
Early termination factors		
VTF (capping enzyme)		
97 kDa	D1R	Morgan <i>et al.</i> (1984)
33 kDa	D12L	Niles <i>et al.</i> (1989)
Nucleoside phosphohydrolase I		
72 kDa	D11L	Deng & Shuman (1998) Christen <i>et al.</i> (1998)
Intermediate transcription factors		
Capping enzyme		
	D1R, D12L	Vos & Stunnenberg (1988)
VITF-1 (RNA polymerase subunit)		
	E4L	Rosales <i>et al.</i> (1994a)
VITF-2		
	Host	Rosales <i>et al.</i> (1994b)
VITF-3 44 kDa		
	A23R	Sanz & Moss (1999)
VITF-3 33 kDa		
	A8R	Sanz & Moss (1999)
YY1		
	Host	Broyles <i>et al.</i> (1999)
Late transcription factors		
VLTF-1 30 kDa		
	G8R	Keck <i>et al.</i> (1990)
VLTF-2 17 kDa		
	A1L	Keck <i>et al.</i> (1990)
VLTF-3 26 kDa		
	A2L	Keck <i>et al.</i> (1990)
VLTF-X		
	A2/B1, RBM3 (Host)	Wright <i>et al.</i> (2001)
Intermediate/late termination factors		
Termination/mRNA release		
	A18R	Xiang <i>et al.</i> (1998)
Termination/mRNA release		
	Host	Lackner & Condit (2000)
Elongation factor		
	G2R	Black & Condit (1996)
Elongation factor J3R		
	J3R	Latner <i>et al.</i> (2000)

non-hydrolysable β - γ bond can be incorporated into RNA chains by the viral RNA polymerase on artificial single-stranded templates, yet do not support transcription from vaccinia virus early promoters (Gershowitz *et al.*, 1978). An explanation for the ATP requirement emerged with the discovery of an ATPase activity associated with ETF (Broyles & Moss, 1988). The ATPase activity is DNA dependent, with little regard for the form or sequence of DNA. Mutations in the conserved ATPase motifs, such as the P loop and DEAH box in ETF, inactivate its transcription factor activity *in vitro* (Li & Broyles, 1995). ATP hydrolysis induces the accelerated dissociation of the ETF-promoter complex (Broyles, 1991).

Taken together, these results suggest a model in which ETF recruits the RNA polymerase to the transcriptional start site but simultaneously presents a steric hindrance to the RNA polymerase because of ETF's DNA contacts on the downstream side of the RNA polymerase (Fig. 2). The release of ETF concurrent with ATP hydrolysis removes the impediment and RNA polymerase could then begin to traverse the template for RNA polymerization.

Another polypeptide specific for early gene transcription is the 95 kDa subunit of the RNA polymerase that is the H4L gene product. About half of the virion-derived RNA

Promoter Class	Consensus Sequence
Early	(A/T) ₈ G(A/T) ₈ N ₁₂ (G/A) ↓
Intermediate	(A/T) ₈ N ₁₂ TAAA(T/A)GG ↓
Late	(A/T) ₆ N ₉ TAAAT

Fig. 1. Comparison of sequence elements in vaccinia virus early, intermediate and late promoters. Numbers following letters indicate the number of continuously repeating residues. 'N' indicates any of the four nucleotides. Arrows indicate transcriptional start sites, referred to as nt +1 in the text. The precise start site in intermediate and late promoters cannot be determined because of RNA polymerase slippage in the oligo(A) tract.

polymerase contains this subunit. Heparin-agarose chromatography is capable of separating RNA polymerase into a population containing, and one deficient in, the H4L subunit (Ahn *et al.*, 1994). Only the fraction containing the H4L subunit supports transcription on early promoter templates *in vitro* (Ahn *et al.*, 1994). In addition, antibodies directed against the H4L polypeptide inhibit early gene transcription (Deng & Shuman, 1994). Taken together, these results imply that H4L may have a role in docking RNA polymerase to the ETF-DNA complex, although this has not been demonstrated directly. Interestingly, H4L has also been shown to have a role in docking several proteins in the transcription elongation complex and is essential for termination of early gene transcription (see below). RNA polymerase deficient for this polypeptide is quite competent to catalyse transcription on late promoter templates (Wright & Coroneos, 1995). Thus, H4L appears to have multiple specific roles in early gene transcription.

Several lines of evidence indicate that the early transcription machinery, complete with RNA modification enzymes, may

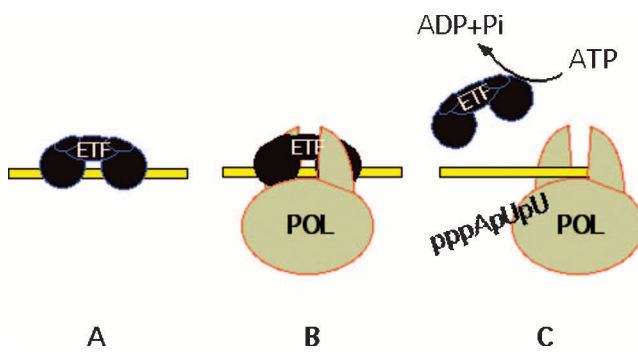


Fig. 2. A model for vaccinia virus early transcription. (A) ETF targets the upstream element of the early promoter. Note that ETF interacts with nucleotides 5' and 3' of the transcriptional initiation site. (B) ETF recruits the RNA polymerase (POL) to the transcriptional initiation site. (C) ATP hydrolysis by ETF releases it from the complex to allow the RNA polymerase to escape from the initiation site.

assemble on early promoters during morphogenesis and assembly into progeny virions. Virion extracts yield a RNA polymerase 'holoenzyme' capable of transcription of early gene templates *in vitro* (Broyles & Moss, 1987). This complex contained ETF, capping enzyme, poly(A) polymerase and the transcription termination factor NPH I (nucleoside phosphohydrolase I). Inhibition of synthesis of the RNA polymerase H4L subunit, thought to dock with ETF, resulted in progeny virions that had normal ETF content but were deficient in RNA polymerase, poly(A) polymerase and capping enzyme (Zhang *et al.*, 1993). Similarly, viruses with ETF having impaired promoter-binding activity packaged reduced levels of ETF, RNA polymerase, capping enzyme, poly(A) polymerase and NPH I within their virion particles (Li *et al.*, 1994). Finally, inhibition of expression of either subunit of ETF caused severe defects in morphogenesis (Hu *et al.*, 1996, 1998). The simplest interpretation of these findings is that the complete early transcription complex is anchored at early promoters during virion assembly through ETF and its complex with the RNA polymerase, and assembly of the transcription complex is an early event in virion morphogenesis. A study by Cassetti *et al.* (1998) seems to contradict the DNA-mediated assembly of vaccinia virus transcription factors. A virus in which the gene A32L product was repressed failed to package significant amounts of DNA (Cassetti *et al.*, 1998). The A32L-deficient virus was, nonetheless, capable of packaging proteins participating in early gene transcription, including ETF. Therefore, factors other than assembly on transcriptional promoters may contribute to the assembly of transcriptional proteins into the virion core.

Intermediate gene transcription

Until recently, vaccinia virus intermediate genes were believed to be few in number. They were uncovered initially by the identification of promoters that required the onset of DNA synthesis but lacked the TAAATG motif at the start site for transcription previously regarded as diagnostic for late promoters (Vos & Stunnenberg, 1988). Intermediate promoters are more prevalent in the vaccinia virus genome than previously appreciated, because many have the TAAATG motif at the start of their open reading frames, which was previously attributed to late gene promoters (X. Liu and S. S. Broyles, unpublished results). The RNA polymerase initiates on this motif within the A triplet (actually on the T triplet on the template strand) and slips repeatedly while attempting to initiate transcription (Bertholet *et al.*, 1987; Schwer *et al.*, 1987). The result is mRNA with a 5' end bearing a heterogeneous oligo(A) tail, averaging about 30 nt in length that is not template encoded. The significance of the 5' oligo(A) tail for mRNA function is not known.

Intermediate promoters are bipartite, having an initiator element at the transcriptional start site and an A-T-rich upstream element (Fig. 1) (Baldick *et al.*, 1992). The initiator element minimally has the sequence TAAAT/A at nt -1 to +4 relative to the first A in the motif (Baldick *et al.*, 1992). Many, but not all, intermediate promoters have the

dinucleotide GG immediately 3' of the TAAAT motif (X. Liu and S. S. Broyles, unpublished results), constituting a binding site for the nuclear transcription factor YinYang1 (YY1) in the form of the sequence TAAATGG. YY1 binds this sequence in the initiator element of the intermediate I1L promoter (Broyles *et al.*, 1999). The I1L promoter was initially described as a late class promoter (Vos & Stunnenberg, 1988) but is now known to be an intermediate class promoter (X. Liu and S. S. Broyles, unpublished results). Replacement of the GG dinucleotide with C residues impaired binding to YY1 *in vitro* and reduced the I1L promoter's activity by about 90% *in vivo*. The co-crystal structure of the DNA-binding domain of YY1 and the sequence AAAATGG showed that the TTT motif, on which the vaccinia virus RNA polymerase must initiate transcription, faces away from the YY1 interface (Houbaviy *et al.*, 1996) and is thus available for engagement. YY1 accumulates in the cytoplasm of vaccinia virus-infected cells, consistent with a role in transcription of the viral genome (Broyles *et al.*, 1999).

Several virus-encoded proteins are required for intermediate gene transcription. *De novo* synthesis of viral RNA polymerase is probably required for vaccinia virus intermediate gene transcription. Temperature-sensitive RNA polymerase mutants are defective for late transcription, suggesting that new RNA polymerase is required for late transcription (Hooda-Dhingra *et al.*, 1989). This observation was reported prior to the discovery of intermediate genes. All RNA polymerase subunit genes whose transcripts have been characterized have early promoters. Therefore, intermediate transcription is likely to require new RNA polymerase also. Either the RNA polymerase brought into the cell by the infecting virion is rendered inactive upon uncoating of the genome and/or is incapable of supporting the burden of RNA synthesis activity necessary for intermediate and late transcription. In addition, the form of RNA polymerase that is most efficient in late gene transcription *in vitro* is the form that lacks the H4L polypeptide (Wright & Coroneos, 1995). The H4L gene is transcribed as a late class gene (Rosel *et al.*, 1986) (although this has not been verified since the discovery of intermediate genes) and, therefore, should not be present during intermediate gene transcription. This means that any transcriptional process that is H4L-dependent is not likely to be functional for intermediate or late gene transcription.

At least four other proteins have been reported from two laboratories to be required for transcription from the I3L intermediate promoter *in vitro*. Vos and co-workers described two factors, ITF-A and ITF-B, that had intermediate transcription factor activity (Vos *et al.*, 1991b). ITF-B is the viral capping enzyme and a fraction containing ITF-A was shown to have promoter DNA-melting activity (Vos *et al.*, 1991a, b). Moss and co-workers have identified the intermediate factors VITF-1, VITF-2 (Rosales *et al.*, 1994a) and VITF-3 (Sanz & Moss, 1998) and confirmed a requirement for the viral capping enzyme in intermediate transcription

(Harris *et al.*, 1993). VITF-1 is the 30 kDa subunit of the viral RNA polymerase (Rosales *et al.*, 1994a). VITF-3 is a heterodimer of the viral A8L and A23R gene products (Sanz & Moss, 1999). VITF-2 was identified in nuclear extracts from uninfected HeLa cells (Rosales *et al.*, 1994b), documenting the first known vaccinia virus transcription factor that is not virus encoded. The identity of the nuclear protein is not known nor is a molecular function for any VITF proteins or capping enzyme. The latter protein likely has a tethering role, linking one of the other factors to the RNA polymerase. The capping enzyme has been reported to be complexed with RNA polymerase in solution (Broyles & Moss, 1987). Whether any of these proteins targets either of the two elements in intermediate promoters is not known.

Protein phosphorylation has been implicated in intermediate transcription through a characterization of vaccinia virus mutants defective for the B1R protein kinase (Kovacs *et al.*, 2001). B1R is a serine/threonine protein kinase (Traktman *et al.*, 1989; Banham & Smith, 1992; Lin *et al.*, 1992) previously characterized as being required for DNA replication (Condit & Motyczka, 1981; Condit *et al.*, 1983). The recent study found that B1R mutants are also defective for intermediate gene transcription but not late gene transcription. These findings suggest that one or more proteins functioning in intermediate transcription may require phosphorylation for function, but the substrate for the kinase that functions in intermediate transcription has not been reported.

Late transcription

Vaccinia virus late promoters also have a bipartite structure with an initiator-like element at the start site for transcription and an A-T-rich upstream element (Fig. 1) (Davison & Moss, 1989a). The initiator element has the nearly invariant sequence TAAAT. Nucleotides downstream of this sequence do not have a role in transcription. The upstream element is closer to the initiator than the intermediate promoters are, being located at about nt -16 to -11 (X. Liu and S. S. Broyles, unpublished results). Like the upstream element of intermediate promoters, the late element seems to tolerate considerable variation in sequence.

As described above, late transcription requires newly synthesized RNA polymerase (Hooda-Dhingra *et al.*, 1989). Three other virus-encoded transcription factors were identified by Moss and colleagues by asking which viral genes must be co-transfected with a reporter gene driven by a vaccinia virus late promoter under conditions where DNA synthesis was inhibited (Keck *et al.*, 1990). G8R, A1L and A2L constituted the minimal set of genes required for late promoter activity. All three are intermediate class genes. No function has been ascribed to any of the three. A yeast two-hybrid screen suggested that the G8R and A1L proteins are interaction partners (McCraith *et al.*, 2000). A fourth factor, the product of the H5L gene, was identified through cell fractionation studies as having transcription stimulatory activity (Kovacs *et al.*, 1994; Kovacs & Moss, 1996). The H5L gene

belongs to the early class of vaccinia virus genes and, hence, would have escaped attention in transfection studies. The H5L protein is a substrate for the B1R protein kinase (Beaud *et al.*, 1995) and an interaction between H5L and B1R was detected in a yeast two-hybrid screen (McCraith *et al.*, 2000), suggesting that protein phosphorylation may have a role in regulating the function of this transcription factor. An effect of phosphorylation on the protein function of H5R has not been reported, nor has its phosphorylation status *in vivo*.

A fifth late transcription factor has been identified by Wright and colleagues, also through cell fractionation (Wright *et al.*, 1998). This factor, called VLTF-X, was initially reported as being virus-induced (Wright & Coroneos, 1993) but, subsequently, was identified in cytoplasmic and nuclear extracts from uninfected HeLa cells (Gunasinghe *et al.*, 1998) and is, therefore, a host protein implicated in vaccinia virus late transcription. Interestingly, this factor co-purified with a DNA-binding activity that demonstrated some specificity for oligo(T)-tract sequences. This is of interest because it has been reported that oligo(T) tracts are functional as an upstream element in a late vaccinia virus promoter (Davison & Moss, 1989a). Thus, it is possible that VLTF-X is responsible for targeting late vaccinia virus promoters as sites of initiation. The transcriptional stimulation activity associated with VLTF-X can be fulfilled by either heterogeneous nuclear riboproteins A2/B1 or RBM3 (Wright *et al.*, 2001). The failure to identify any vaccinia virus-encoded factors with promoter-binding activity prompts speculation that host factors may target the viral promoters, forming a nucleation site for virus-encoded factors that eventually recruit the RNA polymerase to the site of initiation (Fig. 3).

Elongation of transcription

Virtually all DNA-dependent RNA polymerases pause during the elongation phase of transcription (Uptain *et al.*, 1997; Conaway & Conaway, 1999; Gnatt, 2002). The rate at which the enzyme traverses the DNA template is not constant, but rather it can slow dramatically at discrete sequences in the DNA. The pauses are readily detected as less-than full-length transcripts that persist during the course of an *in vitro* transcription experiment. Vaccinia virus RNA polymerase has been shown to pause *in vitro*, especially under conditions

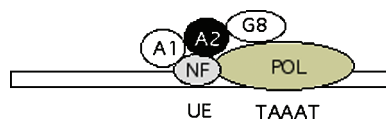


Fig. 3. A hypothetical model to explain the requirement for a host DNA-binding protein in addition to the virus-encoded factors A1, A2 and G8 in late gene transcription. The host nuclear factor (NF) targets its recognition motif in the upstream element of the promoter (UE). The virus-encoded factors facilitate the assembly of the RNA polymerase (POL) at the transcription initiation site (TAAAT).

of limiting nucleotide concentration (Deng & Shuman, 1997). The pauses were reduced by the presence of the virus-encoded NPH I and ATP (Deng & Shuman, 1998). NPH I is a ssDNA-dependent ATPase with nucleic acid helicase motifs in its amino acid sequence. It has been proposed that NPH I weakens the interaction between the RNA polymerase and template DNA to allow movement of the RNA polymerase. It is noted that NPH I has not been demonstrated to have nucleic acid helicase activity.

Two proteins have been implicated in the elongation of transcription on vaccinia virus intermediate and late genes. The G2R and J3R proteins have been suggested to enhance rates of transcription elongation in a manner that likely impacts the ability of the RNA polymerase to terminate and release post-replicative transcripts (see below).

Early gene transcriptional termination

Transcription of vaccinia virus early genes terminates just downstream of open reading frames in response to the sequence TTTTNT (where N is any nucleotide) on the non-template strand of the DNA (Yuen & Moss, 1987). Termination occurs heterogeneously about 30–50 nt downstream of the signal. At least two *trans*-acting factors are required to induce termination and transcript release by the RNA polymerase. The termination signal is actually sensed in the form of the sequence UUUUUNU in the nascent RNA, a conclusion derived from the observation that bromo-UTP specifically inhibits the termination of transcription *in vitro* (Shuman & Moss, 1988). Presumably the RNA polymerase carries the capping enzyme along as it transcribes the template as an elongation complex. As the termination signal in the RNA is extruded from the elongating RNA polymerase, the capping enzyme, by an as yet undefined mechanism, induces the RNA polymerase to cease transcription and release the template. The termination process has been proposed to be the result of a kinetic balance between transcription elongation rates and signalling through the capping enzyme (Deng & Shuman, 1997). Reaction conditions that slow the rate of elongation by the RNA polymerase slow the rate of signalling, thereby shifting termination sites farther downstream. The second factor, NPH I, was identified following the demonstration of an ATP requirement for the termination process (Deng & Shuman, 1998). NPH I is required for termination of transcription and transcript release *in vitro* and for termination of transcription using extracts from cells infected with a NPH I mutant virus (Christen *et al.*, 1998). As described above, NPH I is a ssDNA-dependent ATPase with nucleic acid helicase motifs in its amino acid sequence. Five of the six helicase motifs in NPH I are essential for termination factor activity (Christen *et al.*, 1998). It seems likely that NPH I is the motor that drives dissociation of the transcription elongation complex in response to the signal in RNA. A model for termination of early transcription has been proposed in which RNA polymerase carries capping enzyme and NPH I as an elongation complex (Deng & Shuman, 1998). As the termination signal in the mRNA is

extruded from the RNA polymerase, it contacts the capping enzyme, signalling NPH I to drive release of the transcript through hydrolysis of ATP (Fig. 4). Contact between NPH I and the RNA polymerase is supported by evidence for a requirement for the H4L subunit of the RNA polymerase in termination of transcription (Mohamed & Niles, 2001) and demonstration of direct interaction between NPH I and the H4L polypeptide (Mohamed & Niles, 2000). Thus, the H4L polypeptide seems to be the key specificity factor for virtually all aspects of early gene transcription. It is required for initiation, elongation and termination of early transcripts. Therefore, the RNA polymerase molecules that lack H4L would not be expected to perform any of these processes.

Termination of intermediate and late transcription

Vaccinia virus RNA polymerase appears not to respond to specific termination signals in intermediate and late genes. Northern blotting of these two classes of RNA revealed that their 3' termini are extremely heterogeneous (for example, see Xiang *et al.*, 2000). While the location of termination may not be specified, there is, nevertheless, a growing body of evidence that there is an active mechanism to induce termination of transcription on intermediate and late genes (reviewed by Condit & Niles, 2002). Genetic studies provided the first clues to the concept of active termination of intermediate and late transcription and the proteins participating in the process. Mutants with lesions in the A18R gene form intermediate transcripts that are significantly longer than their counterparts from wild-type virus (Xiang *et al.*, 1998). The A18R polypeptide has classical DNA-dependent nucleic acid helicase motifs and DNA

helicase activity specific for DNA:DNA hybrids less than 20 nt in length (Simpson & Condit, 1995). Extracts from infected cells depleted of A18R by growth of the mutant under non-permissive conditions did not support transcript release in an *in vitro* assay (Lackner & Condit, 2000). Interestingly, purified A18R was incapable of supporting transcript release unless extract from uninfected cells was also provided. The latter result implies a host protein of unknown identity in the termination process.

A role for the vaccinia virus G2R protein in transcription termination was initially revealed by the mapping of mutations conferring dependence on the anti-poxvirus drug isatin β -thiosemicarbazone (IBT) (Meis & Condit, 1991). G2R mutants display a phenotype in which intermediate and late transcripts are shorter than normal (Black & Condit, 1996), just the opposite of that of A18R mutants. The reduced transcript lengths implied that the G2R protein may play a role in promoting transcription elongation by the RNA polymerase. An interrelationship between G2R and A18R was inferred by demonstrating that G2R mutants could act as extragenic suppressors of the A18R mutants (Condit *et al.*, 1996). An interaction between G2R with the late factor H5R was also detected in a yeast two-hybrid screen (McCraith *et al.*, 2000).

A third protein, the product of the J3R gene, has been implicated in intermediate and late transcription termination. Additional IBT-dependent mutants and extragenic suppressors of A18R mutations indicated that J3R mutants are identical in phenotype to those in G2R (Latner *et al.*, 2000; Xiang *et al.*, 2000). Post-replicative transcripts from J3R mutants are truncated at their 3' ends, supporting the conclusion the J3R protein is also a positive elongation factor. A role for J3R in transcription elongation is somewhat surprising because this polypeptide is the viral mRNA 2'-O-methyltransferase (Schnierle *et al.*, 1992) and the stimulatory subunit of the mRNA poly(A) polymerase (Gershon *et al.*, 1991). Mutational analysis showed the mRNA methyltransferase and poly(A) polymerase stimulatory activities to be distinct from the elongation factors' properties (Xiang *et al.*, 2000; Latner *et al.*, 2002). An interaction between the J3R protein and the late transcription stimulatory factor H5R has been documented in two independent studies (Black *et al.*, 1998; McCraith *et al.*, 2000); however, the significance of this complex is not yet apparent. Thus, the termination of post-replicative transcripts appears to result from a dynamic balance between maintaining a transcription elongation complex and promotion of transcript release.

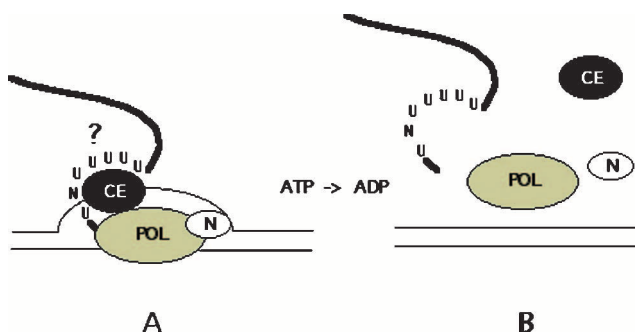


Fig. 4. Model to explain early gene transcriptional termination and transcript release. The transcription elongation complex, consisting of RNA polymerase (POL), capping enzyme (CE) and NPH I (N), drives the movement of the DNA transcription 'bubble' until the capping enzyme receives the UUUUUNU signal in the extruding RNA. The complex pauses and the ssDNA in the non-template strand of the bubble activates the ATPase of NPH I. ATP hydrolysis disrupts the complex to release the RNA. The dissociation of the complex is hypothetical. The interaction between CE and the termination signal in RNA has not been documented directly. Adapted from Deng & Shuman (1998).

Transcript 3'-end processing

The cowpox virus A type inclusion body protein (ati) transcript is a late class mRNA and has the unusual property of terminating at a precise location after the open reading frame (Antczak *et al.*, 1992). The coding sequence terminates at a specific nucleotide by a site-specific ribonucleolytic cleavage and the 3' end is polyadenylated. The

sequence immediately surrounding the cleavage site and another block of sequence 10 nt downstream are essential for the cleavage reaction (Howard *et al.*, 1999). The same 3' end is found on the transcript from the equivalent gene in vaccinia virus (Amegadzie *et al.*, 1992b), indicating that the gene structure is conserved in more than one poxvirus. It is not clear whether 3'-end processing is common in vaccinia virus. Because the first example of 3'-end processing was found in the highly abundant *ati* transcript, it is tempting to speculate that the processing enhances the expression of highly active genes.

CONCLUSION

The emerging picture of vaccinia virus transcription is one in which the majority of proteins required to synthesize a functional mRNA are virus encoded. The early transcription system appears to be performed exclusively by viral proteins and no evidence has been obtained for host functions required for the initiation or termination of early transcripts. The situation is quite different for intermediate and late transcription; key functions for both initiation and termination of intermediate and late transcription appear to be borrowed from the host cell. Why should the intermediate and late systems differ fundamentally from early transcription? Two reasons seem likely: location and timing. The early transcription system is restricted to the confines of the virion core. While the internal structure of the core is a complete enigma, it is likely to be highly ordered. When the core is disassembled, early transcription ceases. Intermediate and late transcription occurs in the cytoplasm in a much more open environment. The timing of transcription may also be a reason for utilizing host proteins. Intermediate and late transcription do not begin until after DNA replication begins. It is possible that host proteins are not recruited to virus replication complexes until sufficient amounts of DNA accumulate in the cytoplasm.

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