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Biochemistry

Vaccinia virus RNA helicase: An essential enzyme related to the DE-H family of RNA-dependent NTPases

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ABSTRACT **Three distinct nucleic acid-dependent ATPases are packaged within infectious vaccinia virus particles; one of these enzymes (nucleoside triphosphate phosphohydrolase II or NPH-II) is activated by single-stranded RNA. Purified NPH-II is now shown to be an NTP-dependent RNA helicase. RNA unwinding requires a divalent cation and any one of the eight common ribo- or deoxyribonucleoside triphosphates. The enzyme acts catalytically to displace an estimated 10-fold molar excess of duplex RNA under *in vitro* reaction conditions. NPH-II binds to single-stranded RNA. Turnover of the bound enzyme is stimulated by and coupled to hydrolysis of NTP. Photocrosslinking of radiolabeled RNA to NPH-II results in label transfer to a single 73-kDa polypeptide. The sedimentation properties of the helicase are consistent with NPH-II being a monomer of this protein. Immunoblotting experiments identify NPH-II as the product of the vaccinia virus I8 gene. The I8-encoded protein displays extensive sequence similarity to members of the DE-H family of RNA-dependent NTPases. Mutations in the NPH-II gene [Fathi, Z. & Condit, R. C. (1991) *Virology* 181, 258–272] define the vaccinia helicase as essential for virus replication *in vivo*. Encapsidation of NPH-II in the virus particle suggests a role for the enzyme in synthesis of early messenger RNAs by the virion-associated transcription machinery.**

Complex transactions of nucleic acids such as DNA replication, transcription, RNA processing, and translation have been shown to be coupled *in vitro* to NTP hydrolysis. The energy of γ-phosphate cleavage may provide mechanical force, elicit conformational changes in proteins or nucleic acids, ensure fidelity in signaling, or specify directionality of movement of protein assemblies along nucleic acids. Many nucleic acid-dependent NTPases have been isolated and characterized. In some cases, the physiological roles of these enzymes can be assigned based on genetic or biochemical experiments. Examples include the prokaryotic transcription termination factor ρ (1), the eukaryotic translation initiation factor EIF4 (2), and the yeast mRNA splicing factors PRP16 and PRP2 (3, 4), all of which display RNA-dependent NTPase activity *in vitro*. Coupling of NTP hydrolysis to protein movement along DNA or RNA strands can result in the disruption of otherwise stable duplexes. Enzymes mediating such reactions are referred to as NTP-dependent helicases (5). Many helicases have been identified, some of which have clearcut roles in initiation of DNA replication, DNA repair, replication fork movement, transcription termination, or translation initiation. Other helicases have been characterized biochemically but have uncertain functions *in vivo*.

Among the first nucleic acid-dependent NTPases to be identified were two enzymes packaged within infectious vaccinia virus particles, referred to as nucleoside triphosphate phosphohydrolases I and II (NPH-I and NPH-II). Both enzymes hydrolyzed ATP to ADP and P_i in the presence of a nucleic acid cofactor (6). NPH-I hydrolyzed only ATP and dATP and was activated by denatured DNA but not RNA. NPH-II cleaved all NTPs in the presence of either denatured RNA or denatured DNA (7). It has long been argued that NPH-I and NPH-II might play roles in the synthesis of vaccinia early mRNAs by the RNA polymerase contained within the vaccinia particle. The possibility that one or both of these enzymes may act as helicases during transcription elongation has been discussed (8, 9), but no direct evidence of helicase activity has been presented. More recently, a third DNA-dependent ATPase from vaccinia virions has been found in association with the viral early transcription factor (VETF). VETF hydrolyzes only ATP and dATP and is activated preferentially by double-stranded (ds) DNA (10). Transcription initiation is coupled to hydrolysis of ATP or dATP at a step subsequent to binding of VETF to the promoter (11). It is suggested that VETF may unwind DNA to form an open promoter complex.

The vaccinia system clearly offers a rich opportunity to explore the biological functions of several distinctive nucleic acid-dependent NTPases. In the present report, I demonstrate that purified NPH-II is an NTP-dependent RNA helicase. The viral gene encoding this enzyme is mapped to an open reading frame previously shown to be essential for vaccinia replication in cell culture (12, 13). The 73-kDa vaccinia RNA helicase is a member of the DE-H family of RNA-dependent NTPases (14) and is extensively similar in primary sequence to the PRP proteins of *Saccharomyces cerevisiae* involved in mRNA splicing (14–16) and to the *Drosophila* maleless (MLE) gene product implicated in dosage compensation of transcription (17).

METHODS

Enzyme Purification. ATPase activity was extracted from vaccinia virions and purified by sequential chromatography on columns of DEAE-cellulose and heparin agarose as described (18, 19). Heparin agarose fractions containing ATPase were pooled, diluted 7-fold with buffer A (50 mM Tris·HCl, pH 8.0/2 mM dithiothreitol/1 mM EDTA/10% glycerol/0.1% Triton X-100), and then applied to an 8-ml column of single-strand DNA agarose. The column was developed with a 120-ml linear gradient of 30–700 mM NaCl in buffer A. NPH-II eluted as a discrete peak at 0.3 M NaCl. Fractions containing NPH-II were pooled, diluted 4-fold with buffer A, and then applied to a 4-ml column of phosphocellulose. The column was developed with a 50-ml linear gradient of 50–600 mM NaCl in buffer A. ATPase activity eluted as a single peak centered at 0.3 M NaCl.

Enzyme Assays. ATPase activity was assayed in the presence of nucleic acid cofactor [either poly(C) or single-stranded M13mp18 DNA] as the release of $^{32}P_i$ from [γ-^{32}P]ATP as described (20). One unit catalyzed hydrolysis of 1 nmol of ATP during 30 min of incubation at 37°C. RNA

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Abbreviations: NPH, nucleoside triphosphate phosphohydrolase; VETF, viral early transcription factor; ds, double-stranded.

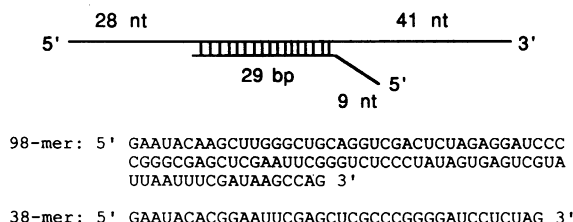
helicase reaction mixtures (20 μl) contained 40 mM Tris·HCl (pH 8.0), 2 mM dithiothreitol, 1 mM $MgCl_2$, 1 mM GTP (or other nucleotides as indicated), 20–50 fmol of ^{32}P-labeled standard dsRNA substrate, and enzyme. After incubation for 15 min at 37°C, reactions were halted by addition of 4 μl of 0.1 M Tris·HCl, pH 7.5/20 mM EDTA/0.5% SDS/50% glycerol. Aliquots (21 μl) were applied to an 8% polyacrylamide gel containing 0.5× TBE (1× TBE = 90 mM Tris/64.6 mM boric acid/2.5 mM EDTA) and electrophoresed at 15 mA constant current. Labeled RNAs were visualized by autoradiographic exposure of the dried gel. The standard helicase substrate (see Fig. 1) was prepared as described (21) and was a generous gift of Chee-Gun Lee and Alejandro Claude of this department.

RESULTS

RNA Helicase Activity from Vaccinia Virions. Of the three nucleic acid-dependent NTPases packaged within infectious vaccinia particles, NPH-II is specifically stimulated by single-stranded RNA (7). Protein fractions obtained during purification of NPH-II from virions were tested for RNA helicase activity *in vitro*. RNA unwinding was manifest as the displacement of a labeled RNA strand from a tailed duplex structure (shown in Fig. 1) of slow electrophoretic mobility to generate the more rapidly migrating free single strand. In the presence of a nucleotide cofactor (GTP), phosphocellulose column fractions containing NPH-II catalyzed quantitative unwinding of the duplex substrate (Fig. 2*A*). Helicase activity paralleled NTPase across the salt gradient elution profile of the phosphocellulose column.

NPH-II sedimented in a glycerol gradient as a single peak of NTPase activity with a sedimentation coefficient of 5.8 S relative to marker proteins centrifuged in parallel. Helicase activity cosedimented with ATPase (Fig. 2*B*). Because NPH-II had been resolved during this purification from other known vaccinia NTPases (not shown) and because the catalytic properties of this purified NTPase were inconsistent with those of the other vaccinia phosphohydrolases (not shown), it was concluded that the helicase activity was intrinsic to NPH-II.

Requirements for Helicase Activity. RNA unwinding was completely dependent on a nucleotide cofactor (Fig. 3). This requirement was satisfied by any one of the eight common ribo- or deoxy-NTPs at 1 mM concentration. Strand displacement also required NTP hydrolysis. AMPPNP and AMPPCP (possessing nonhydrolyzable β—γ bonds) could not substitute for ATP in the helicase reaction; neither analog prevented RNA unwinding when included in reaction mixtures containing ATP (Fig. 3). Only the triphosphate form of nucleotide (e.g., CTP) activated the helicase; CDP and CMP were inactive (Fig. 3). Helicase activity depended completely on the presence of a divalent cation. Among the metals tested at 1 mM concentration, Mg, Co, and Mn were effective cofactors, whereas Ca, Cu, and Zn failed to activate the helicase (Fig. 3).



98-mer: 5' GAAUACAAGCUUGGGCUGCAGGUCGACUCUAGAGGAUCCC
CGGGCGAGCUCGAAUUCGGGUCUCCCUAUAGUGAGUCGUA
UUAAUUUCGAUAAGCCAG 3'

38-mer: 5' GAAUACACGGAAUUCGAGCUCGCCCGGGGAUCCUCUAG 3'

FIG. 1. Structure of the standard dsRNA helicase substrate. The dsRNA substrate consisted of a 98-nucleotide (nt) RNA hybridized to a radiolabeled 38-nt RNA to produce a tailed molecule with a 29-base-pair (bp) duplex region as illustrated. The nucleotide sequences of the two RNA strands are shown.

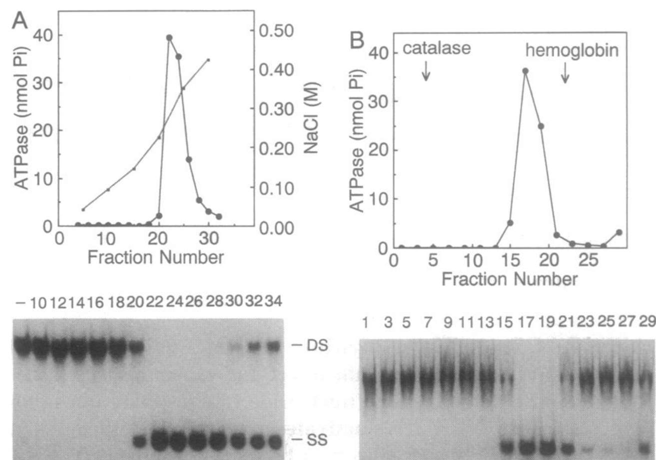


FIG. 2. Copurification of NTPase and helicase activities. (*A Upper*) Aliquots (5 μl) of the indicated phosphocellulose column fractions were assayed for ATPase activity in the presence of poly(C). ATPase (●) is expressed as nmol of $^{32}P_i$ released from ATP during 30 min of incubation at 37°C. The NaCl concentration (- - -) was measured using a conductivity meter. (*Lower*) Aliquots (2 μl) of the indicated column fractions were assayed for RNA unwinding activity in reaction mixtures containing 1 mM GTP and 20 fmol of dsRNA substrate. A control reaction lacking enzyme is shown in lane "–." Incubation was for 15 min at 37°C. The positions of the duplex substrate (DS) and unwound single-stranded RNA (SS) are indicated. (*B Upper*) An aliquot (0.25 ml) of peak phosphocellulose fraction 23 was applied to a 4.7-ml 15–30% glycerol gradient containing 0.5 M NaCl in buffer A and then centrifuged for 18 hr at 55,000 rpm in a Beckman SW55 rotor. Fractions (0.17 ml) were collected from the bottom of the tube. Aliquots (5 μl) of the indicated glycerol gradient fractions were assayed for ATPase activity in the presence of poly(C). Incubation was for 45 min. The positions of reference proteins centrifuged in a parallel gradient are indicated by the arrows. The direction of sedimentation is from right to left. (*Lower*) Aliquots (2 μl) of the indicated gradient fractions were assayed for helicase activity in reaction mixtures containing 1 mM GTP and 50 fmol of dsRNA substrate. Incubation was for 10 min at 37°C.

Kinetics. Aliquots of the helicase reaction mixture were removed at various time points and analyzed by gel electrophoresis. At limiting levels of enzyme (1× in Fig. 4), the amount of displaced strand increased steadily over time, with half the input substrate unwound by about 12 min and with complete unwinding achieved at the 40-min time point. (The range of mobilities of the single-stranded product was caused by the delayed loading of the later time points onto the acrylamide gel, which was electrophoresed continuously during the experiment.) At higher enzyme levels (5× in Fig. 4) the rate of unwinding was accelerated such that the reaction was complete by 2 min. Because the protein concentration of the enzyme fraction was extremely low (estimated at <5 μg/ml), it was not immediately possible to determine the stoichiometry of the helicase reaction. However, if one assumed that unwinding was stoichiometric (i.e., one duplex unwound per NPH-II molecule), then the finding that 1 μl of enzyme unwound 250 fmol of RNA in 40 min (Fig. 4) would mandate a protein concentration of about 20 μg/ml (based on a native M_r of 77,000 for NPH-II; see below). That this value is higher than what was found experimentally suggested that the vaccinia helicase unwound RNA catalytically (this issue is discussed in detail below).

RNA Binding and NTP-Dependent Turnover. The interaction of NPH-II with single-stranded RNA was analyzed by native gel electrophoresis. Binding of the enzyme to a radiolabeled 98-nucleotide RNA (the same species that constituted the longer strand of the standard dsRNA helicase substrate;

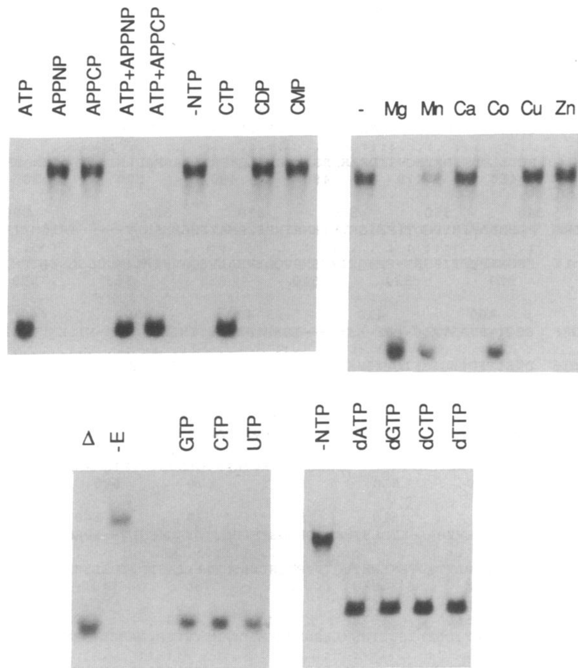


FIG. 3. Requirements for RNA helicase activity. (*Upper Left*) Reaction mixtures contained 1 μl of phosphocellulose fraction 23 (PC-23) and 25 fmol of dsRNA plus nucleotides as indicated at 1 mM concentration each. A control reaction lacking nucleotide is shown in lane "−NTP." Incubation was for 15 min at 37°C. (*Lower Left*) Reaction mixtures contained 2 μl of PC-23 and 20 fmol of dsRNA plus NTPs as indicated at 1 mM concentration each. A control reaction lacking enzyme is shown in lane "−E." Incubation was for 15 min at 37°C. A sample containing labeled RNA substrate that was denatured by heating at 95°C for 4 min followed by cooling on ice is shown in lane "Δ." (*Lower Right*) Reaction mixtures contained 1 μl of PC-23 and 20 fmol of dsRNA plus dNTPs as indicated at 1 mM concentration each. A control reaction lacking nucleotide is shown in lane "−NTP." Incubation was for 15 min at 37°C. (*Upper Right*) Reaction mixtures contained 1 μl of PC-23, 20 fmol of dsRNA, and 1 mM GTP plus divalent cations as indicated at 1 mM concentration each. Mg, Mn, Ca, and Co were added as the chloride salts; Cu and Zn were added as sulfate salts. A control reaction lacking divalent cation is shown in lane "−." Incubation was for 15 min at 37°C.

Fig. 1) was manifest as the appearance of novel species retarded in electrophoretic mobility relative to unbound RNA

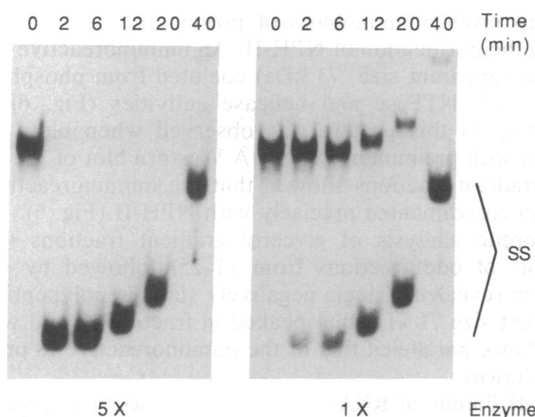


FIG. 4. Time course of RNA unwinding. Reaction mixtures (100 μl) containing 250 fmol of dsRNA and 1 mM GTP received either 5 μl (5×) or 1 μl (1×) of PC-23. At the indicated times aliquots (12 μl) were withdrawn and quenched with stop solution then applied immediately to a continuously running native polyacrylamide gel. An autoradiograph of the gel is shown. The position of the released single-stranded RNA (SS) is indicated.

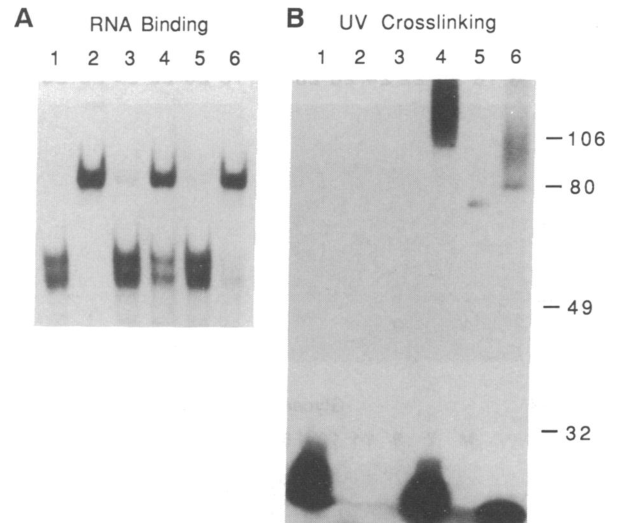


FIG. 5. Binding of NPH-II to RNA. (*A*) Reaction mixtures (20 μl) contained 40 mM Tris·HCl (pH 8.0), 2 mM dithiothreitol, 1 mM $MgCl_2$, 25 fmol of radiolabeled single-stranded RNA [98-mer; prepared as described (21)], and 1 μl of PC-23 (lanes 2–6). A control reaction lacked enzyme (lane 1). Excess unlabeled RNA (500 fmol) was included in one mixture prior to addition of enzyme (lane 3). After incubation for 10 min at 37°C, reaction mixtures were supplemented with 500 fmol of cold RNA (lanes 4–6) and either 1 mM ATP (lane 5) or 1 mM AMPPNP (lane 6). Incubation was continued for an additional 5 min at 37°C; then samples were adjusted to 8% glycerol and electrophoresed through a native 8% polyacrylamide gel containing 0.25× TBE. An autoradiogram of the dried gel is shown. (*B*) Reaction mixtures (20 μl) contained 40 mM Tris·HCl (pH 8.0), 2 mM dithiothreitol, 20 fmol of BrUMP substituted single-stranded RNA (110-mer synthesized with T7 RNA polymerase and labeled using [α-^{32}P]UTP; Y. Luo and S.S., unpublished data) and 1 μl of PC-23 (lanes 3–6). Control reactions lacked enzyme (lanes 1 and 2). After incubation for 10 min at 37°C, samples (lanes 1, 2, and 4–6) were UV irradiated at 4°C for 30 min using a 302-nm transilluminator (UVP model TM-36) situated 5 cm above the samples. The mixtures were either denatured immediately with 1% SDS (lanes 1 and 4) or incubated for 30 min at 37°C in the presence of 7 μg of RNase A (lanes 2, 3, and 5) or 3000 units of RNase T1 (lane 6) prior to SDS addition. Samples were then electrophoresed through a 10% polyacrylamide gel containing 0.1% SDS. An autoradiogram of the dried gel is shown. The positions and sizes (in kDa) of prestained protein markers are indicated.

(Fig. 5*A*, compare lanes 1 and 2). Formation of the protein–RNA complex was inhibited by inclusion of excess unlabeled RNA in the reaction prior to the addition of enzyme (lane 3). Once formed, however, the complex was substantially resistant to dissociation by unlabeled RNA (lane 4). Addition of ATP to the preformed complex markedly stimulated its dissociation in the presence of unlabeled RNA (lane 5). ATP did not have this effect in the absence of excess cold RNA (not shown). The inability of AMPPNP to elicit this effect implied that enzyme turnover required ATP hydrolysis (lane 6); indeed, the protein–RNA complex appeared slightly more resistant to dissociation by cold RNA in the presence of AMPPNP than in the absence of nucleotide (Fig. 5*A*, compare lanes 6 and 4).

The amount of protein–RNA complex detected in the mobility shift assay was proportional to the volume of enzyme fraction added (not shown). By knowing the molar concentration of input RNA and assuming that the shifted species contained one molecule of protein bound to one molecule of RNA, the molar concentration of enzyme (i.e., "RNA binding units") in the peak phosphocellulose fraction was estimated to be 25 pmol/ml (equivalent to 2 μg of protein per ml). Based on this value, the kinetic data of Fig. 4 indicated that at the 1× enzyme level there were 10 molecules

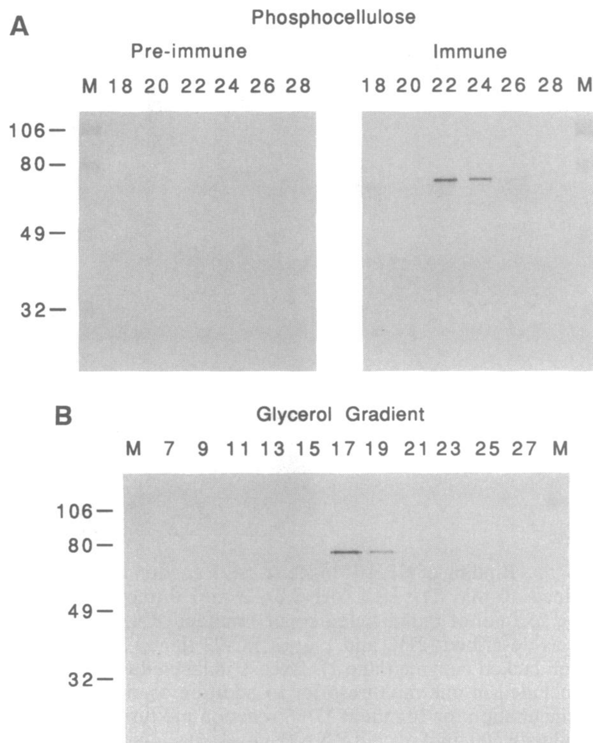


FIG. 6. Western blotting. (*A*) Aliquots (20 μl) of phosphocellulose column fractions were electrophoresed through a 10% polyacrylamide gel containing 0.1% SDS. Polypeptides were transferred electrophoretically to a nitrocellulose membrane that was then blocked in TBST buffer (10 mM Tris·HCl, pH 8/150 mM NaCl/0.05% Tween-20) containing 1% bovine serum albumin. Membranes were incubated for 30 min at room temperature with rabbit serum diluted 1:250 in TBST. After removal of serum and washing with TBST, bound antibodies were localized by incubation with immunoglobulin conjugated with alkaline phosphatase using a ProtoBlot AP system (Promega). Anti-I8 serum and preimmune serum (13) were generous gifts of Richard Condit (University of Florida, Gainesville). (*B*) Aliquots (25 μl) of the indicated glycerol gradient fractions were electrophoresed and immunoblotted with anti-I8 serum as above. The positions and sizes (in kDa) of coelectrophoresed prestained protein markers (lanes M) are indicated.

of RNA displaced per molecule of input enzyme and that, under standard conditions for assay of RNA-dependent ATPase, NPH-II hydrolyzed 160 ATP molecules per enzyme per sec.

Incubation of NPH-II with BrUMP-substituted radiolabeled RNA followed by exposure to UV light resulted in the crosslinking of RNA to protein to yield an SDS-stable adduct seen as a diffuse high molecular weight species during SDS/PAGE (Fig. 5*B*, lane 4). Formation of this covalent complex required enzyme (lane 1). Treatment of the complex with RNase T1 prior to electrophoresis reduced the apparent size of the adduct and converted a portion of the complex to a discrete band of 80 kDa (lane 6), whereas treatment with RNase A (which will more extensively degrade the RNA probe) yielded a sharply defined radiolabeled polypeptide of 73 kDa that was presumed to represent NPH-II (lane 5). Label transfer to the 73-kDa species required UV irradiation (Fig. 5*B*, compare lanes 3 and 5). The sedimentation behavior of NPH-II was consistent with the native enzyme being a monomer of this polypeptide.

Viral Gene Encoding NPH-II. The complete DNA sequence of vaccinia virus (22) reveals the amino acid sequence of 198 virus-encoded polypeptides. Several viral genes encode sequence motifs encountered in known NTP binding proteins and known nucleic acid-dependent NTPases (23). These include the genes for two of the vaccinia ATPases—i.e., the D11 gene encoding NPH-I and the D6 gene encoding the 70-kDa subunit of VETF. Vaccinia gene I8 encodes a 77-kDa polypeptide that (like NPH-II) is packaged within the virus particle (12, 13). The I8 gene contains several sequence motifs characteristic of known RNA-dependent NTPases and, in light of the close correspondence of the predicted size of the I8 polypeptide to that of the NPH-II polypeptide crosslinked to RNA, is a plausible candidate to encode NPH-II.

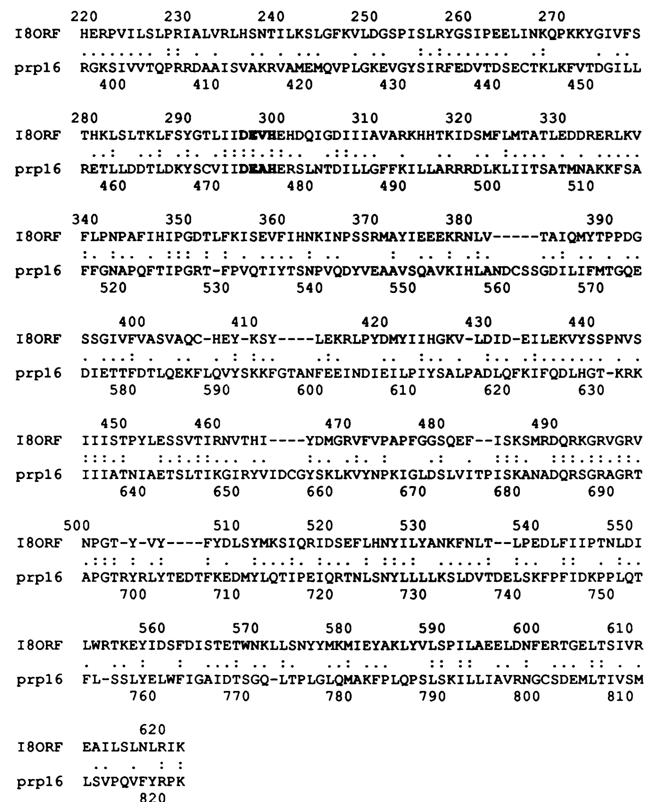


FIG. 7. Sequence similarity between NPH-II (I8ORF) and yeast PRP16. Computer-assisted sequence alignment is shown. Identical amino acids are indicated by a colon (:); conserved amino acids are denoted by a single dot (.). The positions of the amino acid residues are indicated above or below the aligned sequence. The DE-H motif is shown in boldface.

Rabbit antisera specific for the I8 gene product (13) were used to probe immunoblots of protein fractions obtained during the purification of NPH-II. An immunoreactive polypeptide (apparent size, 73 kDa) coeluted from phosphocellulose with NTPase and helicase activities (Fig. 6). No reactivity to this protein was observed when blots were probed with preimmune serum. A Western blot of the glycerol gradient fractions showed that the immunoreactive I8 protein cosedimented precisely with NPH-II (Fig. 6). Electrophoretic analysis of glycerol gradient fractions (25-μl aliquots of odd fractions from 11–25) followed by silver staining revealed a single negatively staining polypeptide of apparent size 71 kDa that peaked in fraction 17 and whose abundance paralleled that of the immunoreactive I8 protein (not shown).

DE-H Family of RNA-Dependent NTPases. The predicted amino acid sequence of the vaccinia helicase/NTPase was compared using the FASTP program (24) to that of other known or presumed members of the helicase/NTPase family. Extensive similarity was found to the yeast splicing factor PRP16, an RNA-dependent NTPase essential for the second step of mRNA splicing (3, 15). The two proteins were aligned with 22% identity over a 404-amino acid region of the vaccinia

enzyme (Fig. 7). This included the signature "DEAH box" characteristic of several yeast mRNA splicing proteins (14) that is represented as the variant sequence DEVH in the vaccinia helicase. The G-GKT nucleotide binding motifs of PRP16 and vaccinia helicase were also strongly conserved but occurred within a protein region of lesser overall similarity and thus are not shown in the alignment in Fig. 7. Vaccinia helicase shows a similar degree of sequence conservation with the PRP2 gene product (not shown), a yeast protein recently shown to be an RNA-dependent NTPase (4), and with the yeast PRP22 protein (not shown), a yeast slicing factor (14) whose NTP hydrolytic properties have not been reported. The vaccinia helicase could also be aligned with the *Drosophila* maleless (MLE) protein (17) over two subregions separated by a nonconserved spacer region (not shown). The first segment includes the nucleotide binding motif and DEVH motif (or the DEIH variant in MLE) and shows 19% identity in a 232-amino acid region; the second segment displays 23% identity over 139 amino acids. The MLE protein, which functions in transcriptional regulation of the *Drosophila* X chromosome and for which no enzymatic activity has yet been reported, is also extensively homologous to the yeast PRP proteins (17). Comparison of vaccinia helicase to the recently determined sequence of human RNA helicase A (a bona fide helicase/RNA-dependent NTPase that has a DEIH motif) revealed conservation over two segments similar to that shown in the alignment to MLE (C. Lee and J. Hurwitz, personal communication).

DISCUSSION

The characterization in 1974 by Paoletti and Moss (6, 7) of vaccinia NPH-II was, to my knowledge, the first demonstration of an RNA-dependent NTPase from any source. In the ensuing years, many other RNA-activated NTPases have been described. Molecular cloning of several genes that encode nucleic-acid dependent ATPases has underscored certain amino acid motifs—e.g., the DEAD and DEAH boxes (25)—whose presence has been invoked in designating as "putative helicases" the protein products of newly cloned genes. The yeast splicing factors PRP16 and PRP2 have been shown to be RNA-dependent NTPases, yet NTP-dependent RNA unwinding by these purified enzymes has not been demonstrated (3, 4). Reexamination of the properties of purified vaccinia NPH-II now reveals this protein to be a virus-encoded NTP-dependent RNA helicase. The predicted amino acid sequence of NPH-II is closely related to three of the yeast PRP proteins, to the *Drosophila* MLE protein, and to one other well-characterized RNA helicase—the human RNA helicase A.

Four distinct thermosensitive (ts) mutants of vaccinia virus have been mapped to the I8 gene, thus defining NPH-II as a protein essential for vaccinia replication (12, 13). Interestingly, the four ts alleles have been localized to single amino acid substitutions (12) within the region of sequence similarity shared by NPH-II and MLE. When the ts viruses are grown in culture at the nonpermissive temperature, they exhibit normal patterns of early and late viral protein synthesis and normal levels of viral DNA replication. Indeed, morphologically normal progeny virions are assembled in the cytoplasm, but these progeny are noninfectious for subsequent rounds of replication at the permissive growth temperature (13). This phenotype is precisely what is expected of mutations affecting a virion-encapsidated protein required for synthesis of early mRNAs by the transcription machinery packaged in the virus core. Studies of early transcription reconstituted *in vitro* suggest that NPH-II is dispensible for initiation, elongation, and termination of transcription by vaccinia RNA polymerase on linear DNA templates (18, 19). The situation during transcription by virions is likely to be more complex, however. In the virion system, newly synthesized mRNAs are extruded actively from the virus core out into the surrounding medium via a poorly understood mechanism that requires high concentrations of hydrolyzable NTP (26, 27). By virtue of its ability to bind to single-stranded RNA and (presumably) to translocate the polynucleotide strand during NTP hydrolysis, NPH-II protein held in a fixed position in the virion could act as an RNA transporter to extrude strands out of the virus core and into the cytoplasm where they are accessible to the translation machinery of the host. Other roles for NPH-II, perhaps in translation, may also pertain since mRNAs made late in infection tend to form stable intermolecular duplexes (28). However, the fact that vaccinia mRNAs are synthesized outside the nucleus and are not spliced tempers any zealous extrapolation to function from the sequence similarity of NPH-II to the yeast PRP proteins. It is possible that NPH-II may be functionally related to the human nuclear RNA helicase A (a protein whose role in RNA metabolism is not yet clear) or to MLE, a protein implicated genetically in transcriptional control.

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