Identification of the Vaccinia Virus Gene Encoding Nucleoside Triphosphate Phosphohydrolase I, a DNA-Dependent ATPase

STEVEN S. BROYLES AND BERNARD MOSS*

Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892

Received 1 December 1986/Accepted 4 February 1987

Vaccinia virus encapsidates a DNA-dependent ATPase known as nucleoside triphosphate phosphohydrolase I (NPH I). A bacteriophage lambda gt11 expression library of poxvirus DNA was screened with antibodies specific for NPH I. Positive clones were used to probe restriction fragments of vaccinia virus genomic DNA to locate the NPH I gene. The identity of the open reading frame (ORF) was confirmed by placing it downstream of a bacteriophage T7 promoter, transcribing the ORF in vitro, and translating the RNA in a reticulocyte lysate. A polypeptide of the correct molecular weight, which was recognized by anti-NPH I antibody, was synthesized. Inspection of the deduced amino acid sequence of the NPH I ORF revealed consensus ATP-binding sites.

The replication of vaccinia virus within the cytoplasm of host cells necessitates an autonomous transcription system. Accordingly, the virus carries within its core all the protein factors necessary to produce mature early mRNA (14). Several virion enzymes, including the DNA-dependent RNA polymerase, mRNA capping enzyme, mRNA 2'-O-methyltransferase, and poly(A) polymerase, have obvious roles in mRNA formation, whereas the functions of others, such as nucleoside triphosphate phosphohydrolases I and II (NPH I and II), DNase, topoisomerase, and protein kinase, are not yet known.

NPH I is a particularly interesting enzyme since it requires single-stranded DNA as a cofactor for hydrolysis of ATP and dATP (18, 19). The appearance of NPH I in infected cells after vaccinia virus replication implies that it is a virus-encoded late protein (17). The following circumstantial evidence suggests that NPH I has a role in the biogenesis of early mRNA. In vitro transcription by permeabilized viral cores (6, 24), solubilized core extracts (7), or partially purified RNA polymerase (2) does not proceed in the presence of ATP analogs with a nonhydrolyzable β - γ bond or γ -thiol. This apparent requirement for β - γ -bond ATP hydrolysis must be the property of a factor which is distinct from the RNA polymerase itself, since purified RNA polymerase does not require hydrolysis of the ATP β - γ bond for non-specific transcription of single-stranded DNA (2, 24). NPH I is the best candidate for this factor since it is the only known ATPase in vaccinia virus that is incapable of hydrolyzing γ -thiol ATP analogs (24). In addition, we have shown that NPH I can exist in a complex with RNA polymerase (2), further supporting a role for NPH I in RNA synthesis. With the possibility that NPH I is a vaccinia transcription factor, it was of interest to identify the gene for this potentially important enzyme.

To obtain DNA probes, antibodies directed against NPH I were used to screen a rabbitpox virus DNA lambda gt11 library (13). Since vaccinia virus and rabbitpox virus are very closely related and share considerable sequence homology (14), we were confident that antibodies specific for vaccinia NPH I would cross-react with the equivalent rabbitpox polypeptide and that the rabbitpox NPH I gene sequence would hybridize to the vaccinia homolog.

The antibody used in these studies was obtained by immunizing a rabbit with highly purified enzyme and was shown to specifically block the activity of NPH I (17). The utility of this antibody preparation for library screening was tested by immunoblot analysis of total deoxycholate-solubilized proteins from vaccinia virions. The antibody preferentially reacted with a polypeptide of 63,000 kilodaltons (kDa), the size expected for NPH I (Fig. 1).

The rabbitpox lambda gt11 library was plated on *Escherichia coli* Y1090 at a density of about 100,000 plaques per 150-mm² dish (28, 29). The recombinant lambda gt11 plaques were screened with immune rabbit immunoglobulin G (IgG) that had been adsorbed with *E. coli* antigens as described previously (29). Positive clones that bound immune IgG were detected by the addition of ¹²⁵I-labeled protein A and autoradiography. Several positive clones were plaque purified four times by the same screening procedure.

FIG. 1. Immunoblot analysis of vaccinia virion proteins and proteins encoded by lambda gt11 recombinants. Total deoxycholate-soluble proteins (21) from purified vaccinia virions (lane 1) or total-cell proteins from *E. coli* lysogenized with lambda gt11 (lane 2) or the recombinants lambda gt11 13A (lane 3), 14A (lane 4), and 15B (lane 5) were electrophoresed on an SDS-polyacrylamide (7.5 to 15%) gradient gel (10). Proteins were transferred to nitrocellulose and probed with IgG prepared from rabbits immunized against NPH I and then by ¹²⁵I-labeled protein A. The positions of NPH I from vaccinia virus and the fusion proteins made in *E. coli* are shown at the left, and the positions of marker polypeptides of indicated size (in kilodaltons [K]) are shown on the right.

* Corresponding author.

FIG. 2. Southern blot analysis of vaccinia virus genome fragments probed with DNA from immunopositive lambda gt11 13A. (A) Vaccinia virus DNA (WR strain) was cleaved with restriction endonuclease *Hind*III (lane 1), *Xho*I (lane 2), or *Sal*I (lane 3) and electrophoresed on a 0.7% agarose gel. DNA was blotted onto nitrocellulose paper which was then probed with 32 P-labeled lambda gt11 13A DNA. Lane 4 contains bacteriophage lambda DNA cleaved with *Hind*III to serve as size markers. Sizes are indicated in kilobase pairs at right. (B) Restriction endonuclease maps for *Hind*III, *Xho*I, and *Sal*I cleavages of vaccinia DNA. Crosshatched boxes mark the fragments which hybridized with immunopositive lambda gt11 recombinant DNAs. Restriction maps are from reference 4.

Three positive clones were chosen for detailed study. Lysogens of bacteriophages 13A, 14A, and 15B were prepared in *E. coli* Y1089 (9). After thermal induction of the lysogens, total-cell proteins were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and immunoreactive polypeptides were identified by blotting followed by probing with anti-NPH I IgG (5). Clones 13A, 14A, and 15B produced immunoreactive polypeptides of 150, 130, and 145 kDa, respectively (Fig. 1). The lambda gt11 vector was constructed so that the inserted DNA would give rise to protein fused to the carboxyl terminus of β -galactosidase. Since the sizes of all three polypeptides are greater than the 114-kDa β -galactosidase, the immunoreactive polypeptides observed in Fig. 1 must be fusion proteins.

DNA was prepared from purified phage (11), labeled with 32 P by nick translation (20), and used to probe blots of electrophoretically separated vaccinia virus DNA segments (25). When vaccinia virus DNA was digested with *Hind*III, *Xho*I, or *Sal*I, phage 13A DNA hybridized with fragments of 16, 23, and 10 kilobase pairs (kb), respectively (Fig. 2), which correspond to the overlapping *Hind*III D, *Xho*I C, and *Sal*I J fragments. DNA from phages 14A and 15B hybridized to the same vaccinia DNA fragments (data not shown). This hybridization pattern indicated that the epitopes which were detected by the anti-NPH I antibodies were encoded within

the right half of the *Hind*III D fragment between the rightmost *Sal*I site and the *Xho*I site.

A recent report (16) indicates the presence of three open reading frames (ORFs; numbers 6, 11, and 14) in the right half of the *Hind*III D fragment that approximate the size expected for the 63-kDa NPH I. To more precisely locate the vaccinia sequences homologous to the phage 13A DNA, a cloned *Hind*III D segment was digested with several different restriction endonucleases and analyzed by Southern blotting and hybridization to labeled phage 13A DNA (Fig. 3). *Bgl*II digestion of *Hind*III-D gave fragments of 5.2 and 3.3 kb which hybridized to the 13A probe. This result confirmed that the right half of the *Hind*III D fragment encoded the NPH I enzyme. The 13A probe hybridized to only the 9.5-kb fragment of the *Pst*I digest of the *Hind*III D segment. No ORF 6 sequences are contained in this fragment, ruling out ORF 6 as a candidate for the NPH I gene. The probe also hybridized to *Eco*RI digestion products of 3.5, 1.2, 0.8, and 0.3 kb and to *Bam*HI fragments of 7.9 and 2.9 kb. These sequences overlap ORFs 6 and 11 but not ORF 14. Significantly, the *Eco*RI 2.4-kb and the *Bam*HI 3.2-kb fragments, which contain ORF 14 in its entirety, showed no hybridization to the probe. Inspection of the regions of the *Hind*III D

FIG. 3. Southern blot analysis of restriction digests of the vaccinia *Hind*III D fragment probed with DNA from immunopositive recombinant lambda gt11 plaques. (A) A plasmid consisting of the vaccinia *Hind*III D fragment in pUC13 was cleaved with *Hind*III (lane 1), *Pst*I (lane 2), *Bgl*II (lane 3), *Eco*RI (lane 4), or *Bam*HI (lane 5). DNA was blotted on nitrocellulose and probed with 32 P-labeled lambda gt11 13A DNA. The positions of lambda-*Hind*III markers are indicated at the right in kilobase pairs. The position of pUC13 DNA which hybridized to lambda gt11 DNA by virtue of β -galactosidase sequences is indicated at the left. (B) Restriction maps for *Pst*I, *Bgl*II, *Eco*RI, and *Bam*HI cleavages of the vaccinia *Hind*III D fragment. The scale in kilobase pairs is shown at the top. Fragments which hybridized with lambda gt11 13A DNA are marked with crosshatched boxes. The locations and orientations of ORFs 6, 11, and 14 are shown below. Other ORFs are omitted for clarity. Restriction cleavage patterns and ORF locations were taken from reference 16, as was the ORF numbering system.

fragment that hybridized to 13A DNA indicated that the boundaries of sequences which hybridized to the probe were between the rightmost *Bam*HI site and the rightmost *Pst*I site (Fig. 3). This agrees well with the determined length of 3 kb for the insert in phage 13A (data not shown). ORF 11 is the only ORF within this region large enough to encode a 63-kDa protein. The right boundary of the insert is located within this ORF as would be expected if a β -galactosidase-ORF 11 fusion protein were produced. All these results are consistent with ORF 11 encoding vaccinia NPH I.

The hybridization results demonstrate that epitopes recognized by the anti-NPH I IgG were encoded by sequences in the region of ORF 11, but the results do not conclusively prove that this ORF encoded the NPH I enzyme. To address this problem, we initially tested the ability of rabbit reticulocyte lysates to synthesize a 63,000-molecular-weight polypeptide (63K polypeptide) when programmed with late RNA which was selected by hybridization to DNA containing only ORF 11. Indeed, we observed this to be the case (data not shown). However, interpretation of this result is not straightforward for the following reason. ORF 11 is flanked by two ORFs which have characteristics consistent with late gene transcription (see below). Other neighboring ORFs also appear to be late genes. Late transcripts are known to have heterogeneous 3' ends which can extend several thousand base pairs beyond the ends of the gene from which they originate (14). As a result, gene mapping by hybridization selection of RNA to DNA located 3' to late genes is subject to potential artifacts. We therefore sought to isolate ORF 11 from the influence of surrounding ORFs. To accomplish this, we placed the ORF 11 gene downstream of a bacteriophage T7 promoter in the pTZ18U vector (12). RNA encoding ORF 11 was produced in vitro with T7 RNA polymerase. Translation of ORF 11 RNA in a rabbit reticulocyte lysate yielded a polypeptide with an apparent molecular weight of 63,000 that was immunoprecipitated by anti-NPH I IgG (Fig. 4). This polypeptide was not synthesized in the absence of added RNA or when RNA was made from a construct containing ORF 11 in the opposite orientation relative to the T7 promoter. These results establish firmly that *Hind*III ORF 11 encoded the 63K NPH I polypeptide.

Two published sequences (16, 27) revealed a single discrepancy near the right end of the *Hind*III D fragment. We confirmed the presence of an additional C at nucleotide 13281 of the *Hind*III D fragment (data not shown). The nucleotide change extends the originally predicted amino-terminal sequence (16) of the NPH I polypeptide to give a protein of 631 amino acids with a molecular weight of 72,319. This is somewhat larger than the 63,000 measured by SDS-polyacrylamide gel electrophoresis of NPH I from vaccinia virions. However, the in vitro translation product of ORF 11 RNA also has a molecular weight of 63,000, suggesting that no proteolytic processing of NPH I occurs after translation in virus-infected cells. The discrepancy between the size of NPH I predicted by ORF 11 and that predicted by SDS-polyacrylamide gel electrophoresis is probably the result of an anomalous electrophoretic mobility.

Walker et al. (26) have identified two conserved sequences found in a wide variety of ATP-binding proteins. The two types of sequences, A and B, may be present either individually or together in the same protein. In some cases, the physical location of these sequences within the nucleotide-binding site is known from structural or biochemical studies. Residues 42 to 71 of vaccinia NPH I show homology to the A-type sequences, and residues 348 to 418 are homologous to B-type sequences (Fig. 5). The NPH I sequences most

FIG. 4. In vitro synthesis of NPH I by using a T7 RNA polymerase transcript. A 2.8-kb *Spe*I fragment containing ORF 11 was placed behind a T7 promoter in the *Sma*I site of the vector pTZ18U. The two plasmid constructs obtained were pSB11, which directs the synthesis of ORF 11 RNA in the correct orientation with 51 base pairs of upstream vaccinia DNA, and pSB12, which has the 2.8-kb *Spe*I insert in the orientation opposite to that in pSB11. The plasmids were cleaved with *Hind*III, and capped RNA was prepared with bacteriophage T7 RNA polymerase and m7GpppGm as previously described (15). RNA was translated in a rabbit reticulocyte lysate in the presence of [³⁵S]methionine, and immunoprecipitations were performed by using protein A-Sepharose (24). Products were electrophoresed on an SDS-polyacrylamide gel. Lanes: 1, proteins synthesized in the presence of pSB11 RNA; 2, anti-NPH I IgG-precipitated proteins from lane 1; 3, preimmune IgG-precipitated proteins from lane 1; 4, proteins synthesized in the presence of pSB12 RNA; 5, anti-NPH I-precipitated proteins from lane 4; 6, molecular weight standards (myosin heavy chain, phosphorylase b, bovine serum albumin, ovalbumin, and carbonic anhydrase) in decreasing mobility.

closely resemble those of the ATPase β subunit and myosin in the A sequence and the ATPases and adenylate kinase in the B sequence. We also noted that the *Hind*III-D ORF 6 predicts a protein in which residues 34 to 63 have a consensus A-type ATP-binding sequence which is very similar to the NPH I sequence. Other homologies of limited extent are found within the amino-terminal halves of the two proteins (data not shown). ORF 6 has the characteristics of a late gene (see below). This fact, together with its size and possible ATP-binding sequence, suggests that ORF6 may encode NPH II.

NPH I is expressed only after the onset of vaccinia DNA replication in infected cells (17) and therefore is a late gene product. The nucleotide sequence around the amino terminus of the NPH I gene is consistent with late gene expression. The initiation codon ATG is immediately preceded by TAA. The TAAATG motif at the initiation codon of vaccinia late genes has been noted by several investigators (1, 18, 23, 27). A late transcriptional start site has been mapped to the 5' terminus of the ORF (27). In addition, the NPH I ORF contains two copies of the sequence ATTTTAT, which has been demonstrated to be a signal for termination of early transcription and is therefore excluded from ORFs of early genes (22; L. Yuen, unpublished observation).

Finally, identification of the gene for NPH I permits studies of the regulation of its expression as well the construction of defined mutations for use in functional studies. It is of interest that two temperature-sensitive complementation groups have been mapped to a DNA fragment which overlaps the amino terminus of NPH I (16). One of the complementation groups may be within the NPH I gene and could be useful for elucidating the function of the enzyme.

	Protein	Residues	
A	Vaccinia NPH I	42-71	F L G L D S M H S L L L F - H E T G V G K T M T T V Y I L K
	Bovine ATPase ^b	144-173	L A P Y A K G G K I G L F - G G A G V G K T V F I M E L I N
	<i>E. coli</i> ATPase ^b	137-165	M C P F A K G G K V G L F - G G A G V G K T V N M M E L I R
	<i>E. coli</i> ATPase ^a	156-184	M I P I G R G Q R E L I I - G D R G T G K T A L A I D A I I
	Adenylate kinase	1-30	M E E K L K K S K I I F V V G G P G S G K G T Q C E K I V Q
	RecA protein	52-84	G A G G L P M G R I V E I Y G P E S S G K T T L T L Q V I A
	Myosin, nematode	156-184	M L Q D H E N Q S M L I T - G E S G A G K T E N T K K V I C
	Myosin, rabbit	165-193	M L T D R E N Q S I L I T - G E S G A G K T V N T K R V I Q
	SV40 T antigen	415-444	M V Y N I P K K R Y W L F K G P I D S G K T T L A A A L E
	Polyoma T antigen	559-588	L T E N V P K R R N I L F R G P V N S G K T Q L A A A L I S
B	Vaccinia NPH I	348-413	V F E P F V N Q S G I E I L L L Y F K V F G I S N I E F S S
	Bovine ATPase ^b	241-270	V A E Y F R D Q E G Q D V L L F I D N I F R F T Q A G S E V
	<i>E. coli</i> ATPase ^b	227-255	M A E K F R D - E G R D V L L F V D N I Y R Y T L A G T E V
	<i>E. coli</i> ATPase ^a	265-293	M G E Y F R D - R G E D A L I I Y D D L S K Q A V A Y R Q I
	ATP/ADP translocase	275-297	S N V L - R G M G G A F V L V L Y D E I K K F V
	Adenylate kinase	102-130	G E E F E R K - I G Q P T L L L Y V D A G P E T M T K R L L
	Phosphofructokinase	85-113	G I E Q L K K - H G I Q G L V V I G G D G S Y Q G A K K L T

FIG. 5. Presence of consensus ATP-binding sites in NPH I. NPH I sequences are aligned with sequences A and B from defined ATP-binding sites of the indicated proteins. Homologous sequences previously identified (26) are enclosed by blocks. Other amino acids which are identical to the vaccinia NPH I sequence are shaded. The alignment of bovine and *E. coli* ATPases, adenylate kinase, RecA protein, myosins, ATP/ADP translocase, and phosphofructokinase is summarized in reference 26. The sequences of simian virus 40 (SV40) and polyomavirus T antigens are from reference 3.

We are grateful to Richard Moyer for the rabbitpox lambda gt11 library; Michael Cashel for bacterial strains and lambda gt11; Michael Merchlinsky for vaccinia DNA and the vaccinia *HindIII*-D clone; Joseph Baldick for synthesis of oligonucleotides; and Patricia Earl, Girish Kotwal, and Andrew Davison for valuable discussions. We also thank Edward Niles and Richard Condit for providing their nucleotide sequence data prior to publication.

LITERATURE CITED

- Bertholet, C., P. Stocco, E. Van Meir, and R. Wittek. 1986. Functional analysis of the 5' flanking sequence of a vaccinia virus late gene. *EMBO J.* 5:1951-1957.
- Broyles, S. S., and B. Moss. 1987. Sedimentation of an RNA polymerase complex from vaccinia virus that specifically initiates and terminates transcription. *Mol. Cell. Biol.* 7:7-14.
- Clerfant, P., and I. Seif. 1984. A common function for polyoma virus large-T and papillomavirus E1 proteins? *Nature (London)* 311:276-279.
- DeFilippes, F. M. 1982. Restriction enzyme mapping of vaccinia virus DNA. *J. Virol.* 43:136-149.
- Erickson, P. F., L. N. Minier, and R. S. Lasher. 1982. Quantitative electrophoretic transfer of polypeptides from SDS polyacrylamide gels to nitrocellulose sheets. *J. Immunol. Methods* 51:241-249.
- Gershowitz, A., R. F. Boone, and B. Moss. 1978. Multiple roles for ATP in the synthesis and processing of mRNA by vaccinia virus: specific inhibitory effects of adenosine (β , γ -imido)triphosphate. *J. Virol.* 27:399-408.
- Golini, F., and J. R. Kates. 1985. A soluble transcription system derived from purified vaccinia virions. *J. Virol.* 53:205-213.
- Hanggi, M., W. Bannwarth, and H. G. Stunnenberg. 1986. Conserved TAAAT motif in vaccinia virus late promoters: overlapping TATA box and site of transcription initiation. *EMBO J.* 5:1071-1076.
- Huynh, T. V., R. A. Young, and R. W. Davis. 1985. Construction and screening cDNA libraries in lambda gt10 and lambda gt11, p. 49-78. In D. M. Glover (ed.), *DNA cloning*, vol. 1. IRL Press, Washington, D.C.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 227:680-685.
- Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. *Molecular cloning: a laboratory manual*, p. 83-85. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Mead, D. A., E. Szczesna-Skorupa, and B. Kemper. 1986. Single stranded DNA "blue" T7 promoter plasmids: a versatile tandem promoter system for cloning and protein engineering. *Protein Eng.* 1:67-74.
- Morrison, D. K., J. K. Carter, and R. W. Moyer. 1985. Isolation and characterization of monoclonal antibodies directed against two subunits of rabbit poxvirus-associated, DNA-directed RNA polymerase. *J. Virol.* 55:670-680.
- Moss, B. 1985. Replication of poxviruses, p. 685-703. In B. Fields, R. M. Chanock, and B. Roizman (ed.), *Virology*. Raven Press, New York.
- Nielsen, D. A., and D. J. Shapiro. 1986. Preparation of capped RNA transcripts using T7 RNA polymerase. *Nucleic Acids Res.*

- 14:5936.
16. Niles, E. G., R. C. Condit, P. Caro, D. Davidson, L. Matusick, and J. Seto. 1986. Nucleotide sequence and genetic map of the 16-kb vaccinia virus *Hind*III D fragment. *Virology* **153**:96–112.
 17. Paoletti, E., N. Cooper, and B. Moss. 1974. Regulation of synthesis of two immunologically distinct nucleic acid-dependent nucleoside triphosphate phosphohydrolases in vaccinia virus-infected HeLa cells. *J. Virol.* **14**:578–586.
 18. Paoletti, E., and B. Moss. 1974. Two nucleic acid-dependent nucleoside triphosphate phosphohydrolases from vaccinia virus: nucleotide substrate and polynucleotide cofactor specificities. *J. Biol. Chem.* **249**:3281–3286.
 19. Paoletti, E., H. Rosemond-Hornbeak, and B. Moss. 1974. Two nucleic acid-dependent nucleoside triphosphate phosphohydrolases from vaccinia: purification and characterization. *J. Biol. Chem.* **249**:3273–3280.
 20. Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg. 1977. Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. *J. Mol. Biol.* **113**:237–251.
 21. Rohrmann, G., and B. Moss. 1985. Transcription of vaccinia virus early genes by a template-dependent soluble extract of purified virions, *J. Virol.* **56**:349–355.
 22. Rohrmann, G., L. Yuen, and B. Moss. 1986. Transcription of vaccinia virus early genes by enzymes isolated from vaccinia virions terminates downstream of a regulatory sequence. *Cell* **46**:1029–1035.
 23. Rosel, J. L., P. L. Earl, J. P. Weir, and B. Moss. 1986. Conserved TAAATG sequence at the transcriptional and translational initiation sites of vaccinia virus late genes deduced by structural and functional analysis of the *Hind*III H genome fragment. *J. Virol.* **60**:436–449.
 24. Shuman, S., E. Spencer, H. Furneaux, and J. Hurwitz. 1980. The role of ATP in *in vitro* vaccinia virus RNA synthesis. *J. Biol. Chem.* **255**:5396–5403.
 25. Southern, E. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98**:503–512.
 26. Walker, J. E., M. Saraste, M. J. Runswick, and N. J. Gay. 1982. Distantly related sequences in the α - and β -subunits of ATP synthetase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. *EMBO J.* **1**:945–951.
 27. Weinrich, S. L., and D. E. Hruby. 1986. A tandemly oriented late gene cluster within the vaccinia virus genome. *Nucleic Acids Res.* **14**:3003–3016.
 28. Young, R. A., and R. W. Davis. 1983. Efficient isolation of genes using antibody probes. *Proc. Natl. Acad. Sci. USA.* **80**:1194–1198.
 29. Young, R. A., and R. W. Davis. 1983. Yeast RNA polymerase II genes: isolation with antibody probes. *Science* **222**:778–782.