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Fine Structure Mapping of Five Temperature-Sensitive Mutants in the 22- and 147-Kilodalton Subunits of Vaccinia Virus DNA-Dependent RNA Polymerase

CAROL L. THOMPSON, URVASHI HOODA-DHINGRA, AND RICHARD C. CONDIT*

Department of Biochemistry, State University of New York at Buffalo, 140 Farber Hall, Buffalo, New York 14214

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We have mapped the temperature-sensitive (*ts*) lesions of three mutants, *ts*51, *ts*53, and *ts*65, and two other mutants, *ts*7 and *ts*20, to regions on the vaccinia virus genome that encode the 147- and 22-kilodalton subunits of the viral DNA-dependent RNA polymerase, respectively. Plasmid and bacteriophage clones from the *Hin*dIII J region and the region spanning the *Hin*dIII J-H junction were used in marker rescue experiments to map the mutations. Sequence analysis of the region encoding the 22-kilodalton subunit in the wild-type, *ts*7, and *ts*20 viruses revealed a single base change in the mutants compared with that in the wild-type virus. The identification of these RNA polymerase mutants provides us with tools to understand transcription and its regulation in vaccinia virus.

The production of mature eucaryotic mRNAs requires sequence-specific initiation of transcription, template-directed polymerization, specific processing events, and modification of the 5′ and 3′ ends of the resulting mRNA molecules. All the data obtained for eucaryotic transcription systems are derived either from purely biochemical approaches or from the development of reverse genetics. Despite the large volume of information available, the mechanism of transcription and its regulation in eucaryotes is poorly understood. The isolation and characterization of conditional lethal mutations in genes involved in transcription would facilitate our understanding of eucaryotic transcription. However, the large genome size and the complexity of the arrangement of eucaryotic genes have made it difficult to employ a genetic approach in higher eucaryotes. We have chosen vaccinia virus as a system for studying transcriptional regulation because its genome size is smaller and less complex than the genomes of higher eucaryotes.

Vaccinia virus is a double-stranded DNA-containing virus which replicates in the cytoplasm of the host cell. It encodes all of the enzymes essential for carrying out viral DNA and RNA synthesis (20). Vaccinia virus gene expression is temporally regulated. Expression of early viral genes begins immediately after infection and continues until DNA replication. When DNA replication begins, expression of most early genes ceases and late gene expression is initiated. The early and late viral genes that have been characterized to date possess specific *cis*-acting sequences at their 5′ ends which are essential for transcription by the viral RNA polymerase (4, 7, 10, 18, 32, 33). Transcription of early genes terminates in response to a specific DNA sequence, therefore the early transcripts are of a distinct size (24, 34). In contrast, transcription of late genes does not terminate in a sequence-specific fashion, resulting in late transcripts which are heterogeneous in size (16). The regulatory mechanisms whereby the viral RNA polymerase recognizes the transcription initiation and termination signals and switches from early to late gene expression are poorly understood. Identification of conditional lethal mutations in the virus-encoded RNA polymerase and the subsequent characterization of the mutants might help us understand some of these processes.

The virus-specific DNA-dependent RNA polymerase is a multisubunit enzyme (2, 21, 28) which has immunologic cross-reactivity (19) and amino acid homology (5) with cellular RNA polymerase II. Biochemical experiments have mapped nine subunits of the vaccinia virus RNA polymerase to various regions on the genome (15, 19). Although further mapping is required for most of the viral RNA polymerase subunits, detailed transcriptional (1, 16, 17, 25) and translational (1, 15, 19, 25) analysis and sequence data (5, 23, 25, 31) of a region encoding two of the subunits have been published. The data show that 60% of the sequence that included the *Hin*dIII J fragment and the left end of the *Hin*dIII H fragment is utilized for a small (22-kilodaltons [kDa]) subunit and a large (147-kDa) subunit of the viral RNA polymerase (5). We have previously reported the isolation and preliminary characterization of 65 temperature-sensitive (*ts*) mutants of vaccinia virus (8, 9), and most of the *ts* lesions in the collection have been mapped on the viral genome at low resolution (9, 29). In this report, we report the mapping and sequence analysis of two *ts* mutations, *ts*7 and *ts*20, which we show lie within the gene encoding the 22-kDa subunit of the viral RNA polymerase. We have also identified three *ts* mutations, *ts*51, *ts*53, and *ts*65, as mutations in the gene encoding the 147-kDa subunit of the viral RNA polymerase. The phenotypic characterization of these mutants is presented in the accompanying report (14).

MATERIALS AND METHODS

Cells and viruses. BSC40 cells, wild-type (wt) vaccinia virus strain WR, mutant viruses *ts*7, *ts*20, *ts*51, *ts*53, and *ts*65, the conditions for their growth, infection, and plaque assay have previously been described (8, 9).

Molecular cloning. The map positions of all the cosmid and plasmid clones used for the marker rescue experiments in this report are shown in Fig. 1. The method of construction and growth of the cosmid clones have been previously described (29). The plasmid clones were constructed as described below and were used to transform *Escherichia coli* JM83.

pKA. The cosmid pWR67-98 was cleaved with the restric-

* Corresponding author.

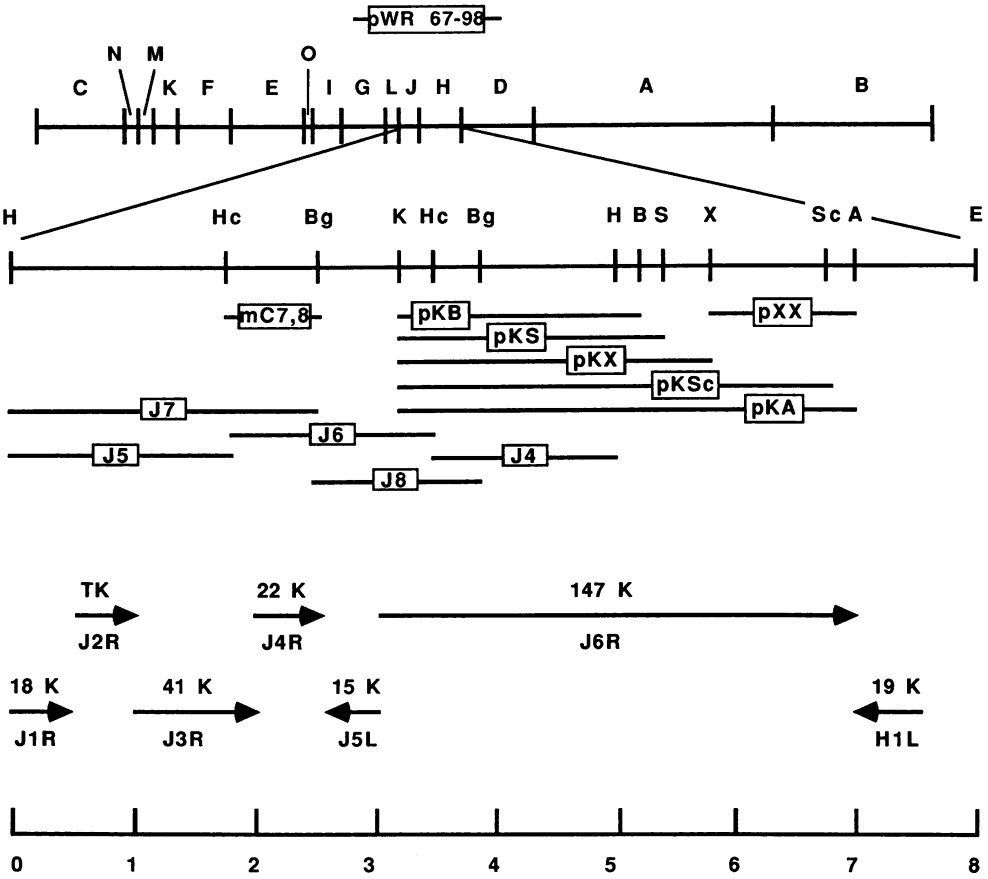

FIG. 1. Diagram of restriction endonuclease sites, ORFs, and map positions of recombinant clones from the *Hin*dIII J-H region of vaccinia virus genome. From the top down, the following are shown. (i) Map position of the cosmid clone pWR67-98. (ii) *Hin*dIII restriction map of the vaccinia virus genome. (iii) Restriction map of *Hin*dIII-J and the left end of *Hin*dIII-H up to the first *Eco*RI site in *Hin*dIII-H. Restriction endonuclease site abbreviations: A, *Aha*III; B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; Hc, *Hin*cII; K, *Kpn*I; S, *Sal*I; Sc, *Sca*I; X, *Xba*I. Other restriction endonucleases cut within this region and some of the above enzymes cut at additional sites in this region, but only those used in the construction of recombinant clones are shown. (iv) Lines with labeled boxes indicate the map positions of clones used in subsequent marker experiments. (v) Physical location of ORFs in the *Hin*dIII J-H region. The arrows indicate the direction of translation. Identifying characteristics of each ORF are given above each arrow. TK, Thymidine kinase; other designations indicate predicted masses of the protein products (in kilodaltons). Alphanumeric designations below the arrows are formal names of the ORFs. For example, J4R is the fourth complete ORF from the left end of *Hin*dIII-J and is translated rightward. (vi) A scale in kilobases.

tion enzymes *Kpn*I and *Aha*III and a 3.8-kilobase (kb) *Kpn*I-*Aha*III fragment was purified by gel electrophoresis and "freeze phenol" extraction (3). This fragment was ligated to *Kpn*I- and *Sma*I-cleaved pUC19.

pKB. pKA was cleaved with the restriction enzyme *Bam*HI. A 4.7-kb *Bam*HI-*Bam*HI fragment was purified by gel electrophoresis as described above and recircularized by ligation.

pKS. pKA was cleaved with the restriction enzyme *Sal*I. A 4.9-kb *Sal*I-*Sal*I fragment was purified by gel electrophoresis and recircularized by ligation.

pXX. The plasmid clone pKA was cleaved with the restriction enzyme *Xba*I. A 1.2-kb *Xba*I-*Xba*I fragment was isolated by using the method described above. This fragment was ligated to the vector pUC19 which had been cleaved with the restriction enzyme *Xba*I and treated with calf intestinal phosphatase.

pKSc. The plasmid clone pKA was cleaved with the restriction enzymes *Kpn*I and *Sca*I. All the resulting fragments were then end filled by using the Klenow fragment of *E. coli* DNA polymerase I. A 3.5-kb fragment was then purified by gel electrophoresis. This end-filled *Kpn*I-*Sca*I fragment was ligated to *Sma*I-cut, phosphatase-treated pUC19.

pKX. pKA was cleaved with the restriction enzyme *Xba*I. A 5.3-kb *Xba*I-*Xba*I fragment was purified by using the method described above and recircularized by ligation.

Plasmid clones J4, J5, J6, J7, and J8 (Fig. 1) (12) were obtained from Jerry Weir.

mC7 and mC8. Plasmid DNA J6 was cleaved with *Hin*cII and *Bgl*II, and *Bgl*II 5′ overhangs were filled by treatment with Klenow fragment in the presence of four deoxynucleoside triphosphates, and the 700-base-pair (bp) *Hin*cII-*Bgl*II fragment was purified by electrophoresis in low-melting-temperature agarose and ligated to *Sma*I-cleaved, phosphatase-treated M13mp18 replicative-form (RF) vector DNA. Transformations were done on *E. coli* JM101. Two bacteriophage clones (mC7 and mC8) were isolated, each containing the vaccinia virus *Hin*cII-*Bgl*II fragment (Fig. 1) inserted into the *Sma*I site in M13mp18, but in opposite orientations relative to each other. Clone mC7 is oriented

with the *Hin*cII end of the vaccinia virus DNA insert closest to the *Hin*dIII site in the M13mp18 polylinker.

Deletion subclones of mC7 and mC8 were constructed by using the exonuclease III procedure described by Henikoff (13). M13 polylinker sites *Sal*I and *Pst*I were used to linearize mC7 or mC8 RF DNA prior to exonuclease III treatment. Several deletion clones were obtained and their deletion endpoints were determined by dideoxy sequencing (26). mC8 subclones 22c, 19b, and 13a and mC7 subclones 1a, 6a, and 11c (see Fig. 4) were selected for marker rescue experiments.

DNA sequence analysis. For use in determining the sequences of the mutations in *ts*7 and *ts*20, the 1.7-kb *Hin*cII subfragment of *Hin*dIII-J was cloned from wt, *ts*7, and *ts*20 viral DNA into M13 vectors. Vaccinia virus DNAs from wt, *ts*7, and *ts*20 were cleaved with *Hin*dIII, the 5-kb J fragment was purified by gel electrophoresis and ligated to *Hin*dIII-cleaved, phosphatase-treated pUC13. *E. coli* TB1 (Bethesda Research Laboratories, Inc.) was used as a host for transformations. *Hin*dIII J fragment clones from each virus were cleaved with *Hin*cII, a 1.7-kb fragment was purified by gel electrophoresis and ligated to *Sma*I-cleaved, phosphatase-treated M13mp18 RF DNA, and the ligations were used to transform *E. coli* JM101. Five phage clones were isolated which contain inserts of the 1.7-kb vaccinia virus *Hin*cII fragment, with the vaccinia virus *Bgl*II and *Kpn*I sites oriented with respect to the M13mp18 polylinker sites *Eco*RI and *Hin*dIII as follows: wt-1 and *ts*20-1 have the order *Eco*RI-*Kpn*I-*Bgl*II-*Hin*dIII; wt-5, *ts*20-4, and *ts*7-10 have the order *Eco*RI-*Bgl*II-*Kpn*I-*Hin*dIII. Phage clone *ts*7-10I, which has the reverse orientation of *ts*7-10 was prepared as follows: RF DNA from clone *ts*7-10 was cleaved with *Hin*dIII and *Eco*RI, the 1.7-kb viral DNA-containing fragment ws purified by gel electrophoresis, ligated to *Eco*RI, *Hin*dIII-cleaved M13mp19 RF DNA, and the ligations were used to transform *E. coli* JM101.

TABLE 1. Results of marker rescue experiments

DNA added	No. of plaques/dish		
	*ts*51	*ts*53	*ts*65
None	0	0	0
pWR67-98	1,000	500	1,000
*Hin*dIII-J	14	0	0
*Hin*dIII-H	0	2	1,000
J4	0	ND[a]	ND
J6	2	ND	ND
J8	17	ND	ND
pKA	1,000	500	150
pKB	1,000	12	0
pKS	400	35	0
pKX	200	300	0
pKSc	ND	ND	0
pXX	ND	ND	75

[a] Not determined.

Synthetic oligonucleotides. Four synthetic oligonucleotides were obtained for use as primers in sequencing: p1, 5′ TATAACATCGTATCTTC 3′; p2, 5′ ATTGATTAATCAG CGAC 3′; p3, 5′ ACCACATCTATGTATAG 3′; and p4, 5′

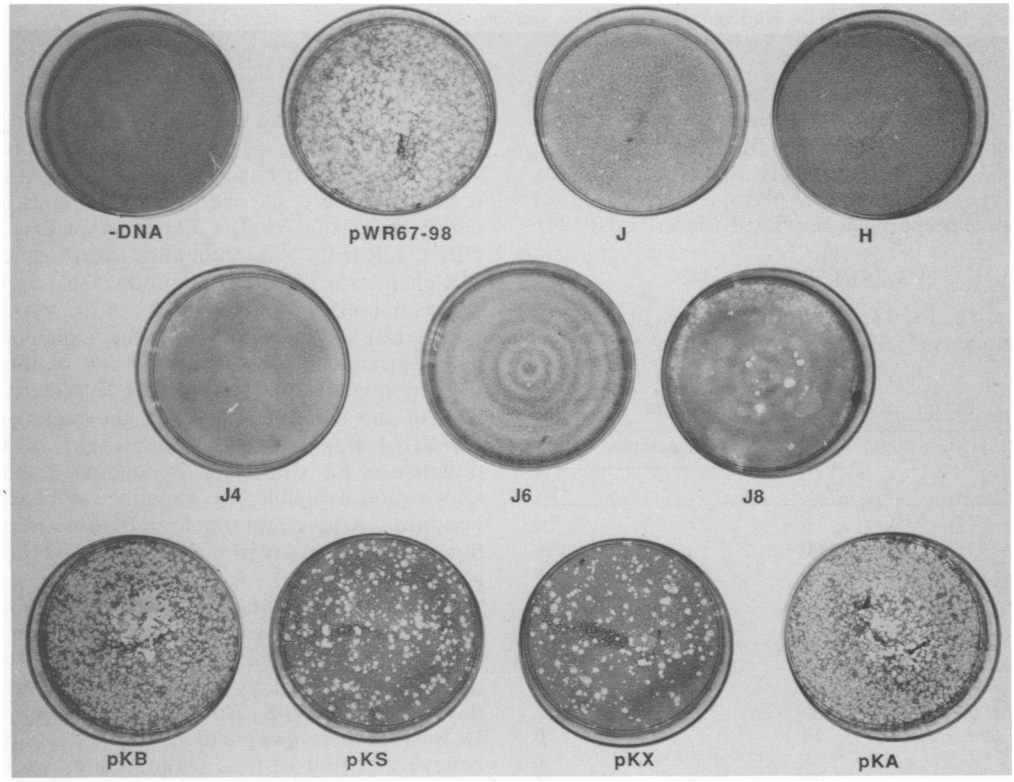

FIG. 2. Marker rescue of *ts*51. Cells were infected with *ts*51 and then transfected with cloned DNA fragments, as described previously (29). The letter below each dish indicates the identity of the DNA added. The map positions of the recombinant DNA fragments used are shown in Fig. 1. Dishes were incubated at 40°C for 4 days and then stained with crystal violet. The presence of virus plaques on any dish indicates that rescue has taken place. A control (−DNA) is also shown.

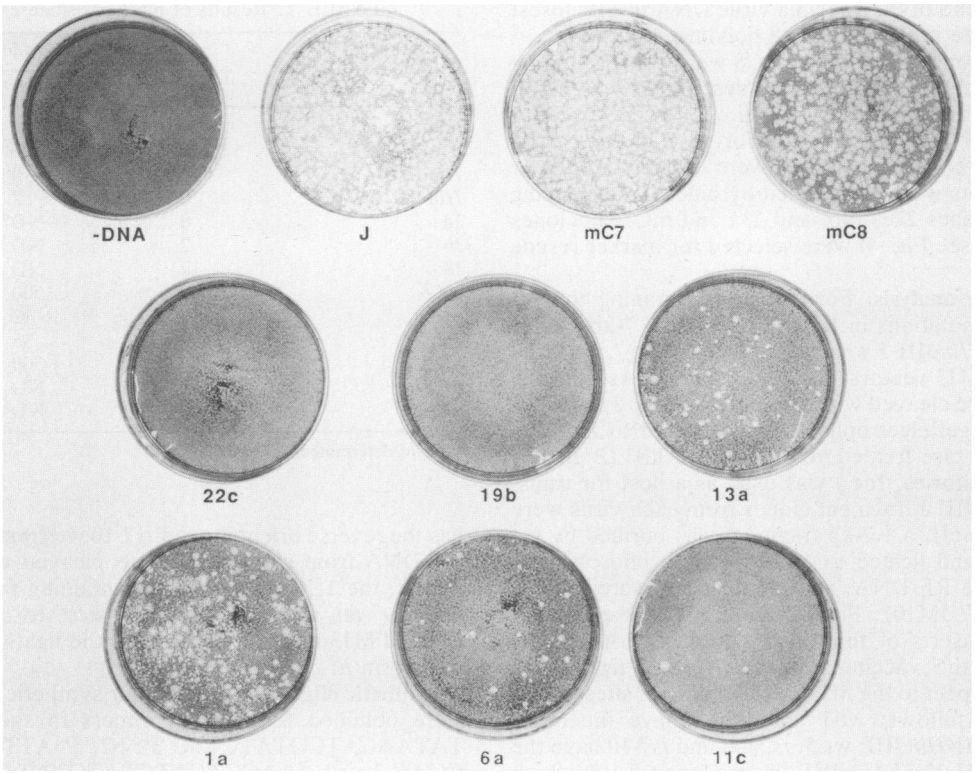

FIG. 3. Marker rescue of *ts*20. Cells were infected with *ts*20 and then transfected with recombinant DNA fragments as described previously (27, 29). The letter below each dish indicates the identity of the DNA added. The map positions of the recombinant DNA fragments are shown in Fig. 1 and 4. Dishes were incubated at 40°C for 4 days and then stained wtih crystal violet. The presence of plaques on any dish indicates that rescue has taken place. A control (−DNA) is also shown.

ACGTTTCTATTTATTAC 3′. The binding sites for these primers are indicated below (see Fig. 5).

Marker rescue. Marker rescue was done as previously described (27, 29). The cosmid and plasmid DNAs used for marker rescue were prepared as previously described (6, 29).

RESULTS

Transcriptional (1, 16, 17, 25), translational (15, 19, 25), and sequence analysis (5, 23, 25, 31) of a central conserved region of the vaccinia virus genome has been performed, and the functional roles of a few of the gene products encoded within this region have been described. In the *Hin*dIII J region, there are six open reading frames (ORFs), one of which spans the *Hin*dIII J-H junction (Fig. 1). Of the six ORFs, J2R is the viral thymidine kinase gene (1, 30, 32) and J4R encodes a 22-kDa gene product which has been identified as one of the small subunits of the vaccinia virus RNA polymerase (5, 15). ORF J6R, which spans the *Hin*dIII J-H junction, encodes a 147-kDa subunit of the vaccinia virus RNA polymerase (Fig. 1) (5, 15, 19). Thus, approximately 60% of the coding capacity in the region comprising the *Hin*dIII J fragment and the left end of the *Hin*dIII H fragment is for viral RNA polymerase subunits. With this information available, we examined our collection of temperature-sensitive mutants to determine whether any mutations were located within this region of the vaccinia virus genome.

TABLE 2. Results of marker rescue experiments

DNA added	No. of plaques/dish	
	*ts*7	*ts*20
None	0	0
J	2,000	1,000
J4	0	0
J5	1	0
J6	75	150
J7	20	100
J8	1	0
mC7	2,000	2,000
mC8	300	500
22c	10	0
19b	1	0
13a	20	35
11c	15	6
6a	6	30
1a	200	100

The *ts* lesions of the 65 mutants in our collection had been grossly mapped to various regions on the vaccinia virus genome (9, 29). The data showed that we had five *ts* mutations, *ts*7, *ts*20, *ts*45, *ts*53, and *ts*65, which mapped to the region comprising *Hin*dIII-J and the left end of *Hin*dIII-H. Besides their map positions, we had additional reasons to believe that four of these *ts* mutations were lesions in the viral RNA polymerase subunits. (i) Preliminary phenotypic characterization of *ts*7, *ts*20, and *ts*53 had shown that these mutants were defective in the expression of late genes at the nonpermissive temperature, which suggested a possible de-

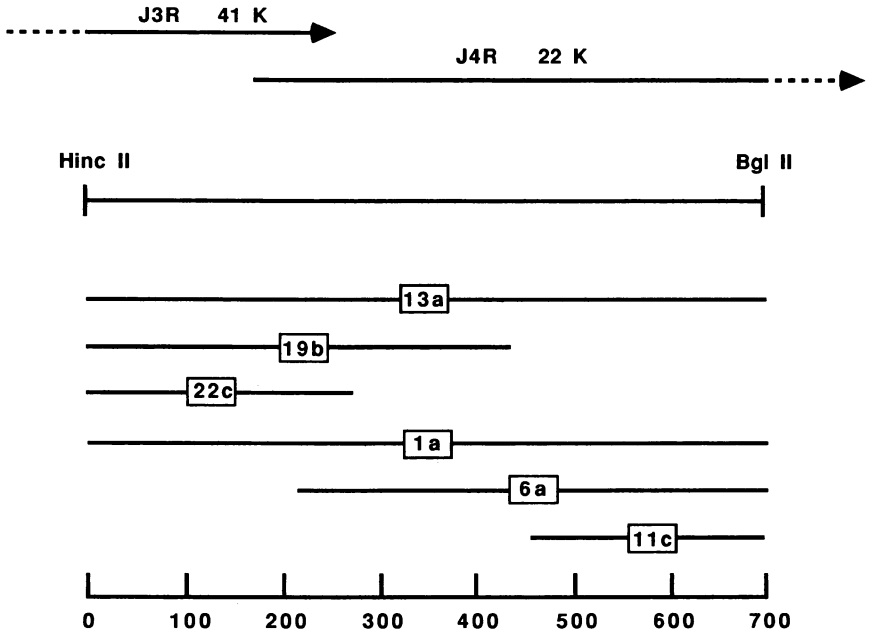

FIG. 4. Map of the *Hinc*II-*Bgl*II region of *Hin*dIII-J. From the top down, the following are shown. (i) Map positions of ORFs J3R and J4R. Arrowheads indicate the direction of translation, and dotted lines indicate that the ORFs continue outside of the region shown. (ii) *Hinc*II and *Bgl*II restriction sites. (iii) Lines with labeled boxes indicate the map position of DNA contained in six deletion subclones of the *Hinc*II-*Bgl*II fragment (see Materials and Methods). (iv) A scale in base pairs. See the legend to Fig. 1 for other details.

fect in the viral transcriptional machinery (8, 9). (ii) Although it had been reported that *ts*65 is normal in its overall gene expression at the nonpermissive temperature (9), its map position made it a likely candidate for being an RNA polymerase mutant. *ts*65 had been mapped to the left end of the *Hin*dIII H fragment (29), and sequence analysis showed that 95% of this region encoded the 147-kDa subunit of the viral RNA polymerase (5). (iii) Although *ts*51 had not been mapped to the same region as that of *ts*65, it has been shown to be a member of the same complementation group as that of *ts*65 (9). Thus, on the basis of either their map positions, their phenotypic characteristics, or their complementation properties, it seemed likely that *ts*7, *ts*20, *ts*51, *ts*53, and *ts*65 contained mutations in viral RNA polymerase subunits. We therefore decided to map the fine structures of these mutations.

Mutations in the 147-kDa RNA polymerase subunit. In a preliminary attempt to assess the map position of *ts*53 relative to other mutants in the *Hin*dIII L, J, and H region, recombination analysis was done between *ts*53 and *ts*65 and between *ts*53 and *ts*7. Very low recombination frequencies were obtained when *ts*53 was crossed with *ts*65 and higher recombination frequencies were obtained when *ts*7 was crossed with *ts*53 (data not shown). Qualitative and quantitative complementation analysis indicated that the low recombination frequency between *ts*53 and *ts*65 resulted from noncomplementation between *ts*53 and *ts*65. These results are consistent with extensive intragenic and intergenic recombination analysis of other vaccinia virus mutants (J. Seto, Z. Fathi, L. Celenza, R. C. Condit, and E. Niles, unpublished observations). On the basis of these results, *ts*53 was assigned to the same complementation group as that of *ts*65 and *ts*51. This complementation group had previously been mapped to the right of *ts*7 (9).

The fine structures of *ts*51, *ts*53, and *ts*65 were mapped, using marker rescue. The restriction map of the *Hin*dIII J and H region and the recombinant DNA fragments used in the marker rescue experiments are shown in Fig. 1. The results of the marker rescue experiments are shown in Table 1 and Fig. 2. All three members of this group were rescued by clone pKA. This places the three mutations between the unique *Kpn*I site in *Hin*dIII-J and the first *Aha*III site in *Hin*dIII-H (see Fig. 1 and 7). Two transcriptionally active ORFs have been mapped to this region. A 4,350-nucleotide ORF (J6R) that encodes one of the large subunits of the RNA polymerase spans the *Hin*dIII J-H junction. A second ORF (H1L) that encodes a 19-kDa polypeptide of unidentified function overlaps the 3′ end of J6R by 42 nucleotides (5) (see Fig. 1 and 7).

In order to unequivocally map *ts*51, *ts*53, and *ts*65 to the large subunit of RNA polymerase, subclones from the *Hin*dIII J-H region were constructed (Fig. 1) and used for marker rescue. The marker rescue results for *ts*51 are shown in Fig. 2. *ts*51 was rescued with *Hin*dIII-J, J8, and pKB. J4 and J6 did not rescue *ts*51. The failure of *ts*51 to be rescued efficiently with either J6 or J4 may indicate that *ts*51 maps very near the *Hinc*II site in J8. On the basis of these results, we placed *ts*51 in the 1,300-bp region to the right of the *Kpn*I site in *Hin*dIII-J (see Fig. 7). *ts*53 was rescued with pKB, pKS, and pKX (Table 1). *ts*53 therefore maps to a 2-kb region between the *Kpn*I site in *Hin*dIII-J and the *Bam*HI site in *Hin*dIII-H (see Fig. 7). Only the transcript that encodes the 147-kDa subunit of RNA polymerase has been mapped to this region (see Fig. 1 and 7). Thus, *ts*51 and *ts*53 are mutations in the 147-kDa subunit of vaccinia virus DNA-dependent RNA polymerase.

*ts*65 was rescued with the *Hin*dIII H fragment and clone pXX. *ts*65 was not rescued with pKB, pKS, pKX, or pKSc (Table 1). *ts*65 therefore lies in a 1.2-kb *Xba*I-*Aha*III fragment in *Hin*dIII-H (see Fig. 7). This region contains 42 nucleotides of the 19-kDa ORF, H1L. Since *ts*51 and *ts*53 unequivocally map to the large subunit of the RNA polymer-

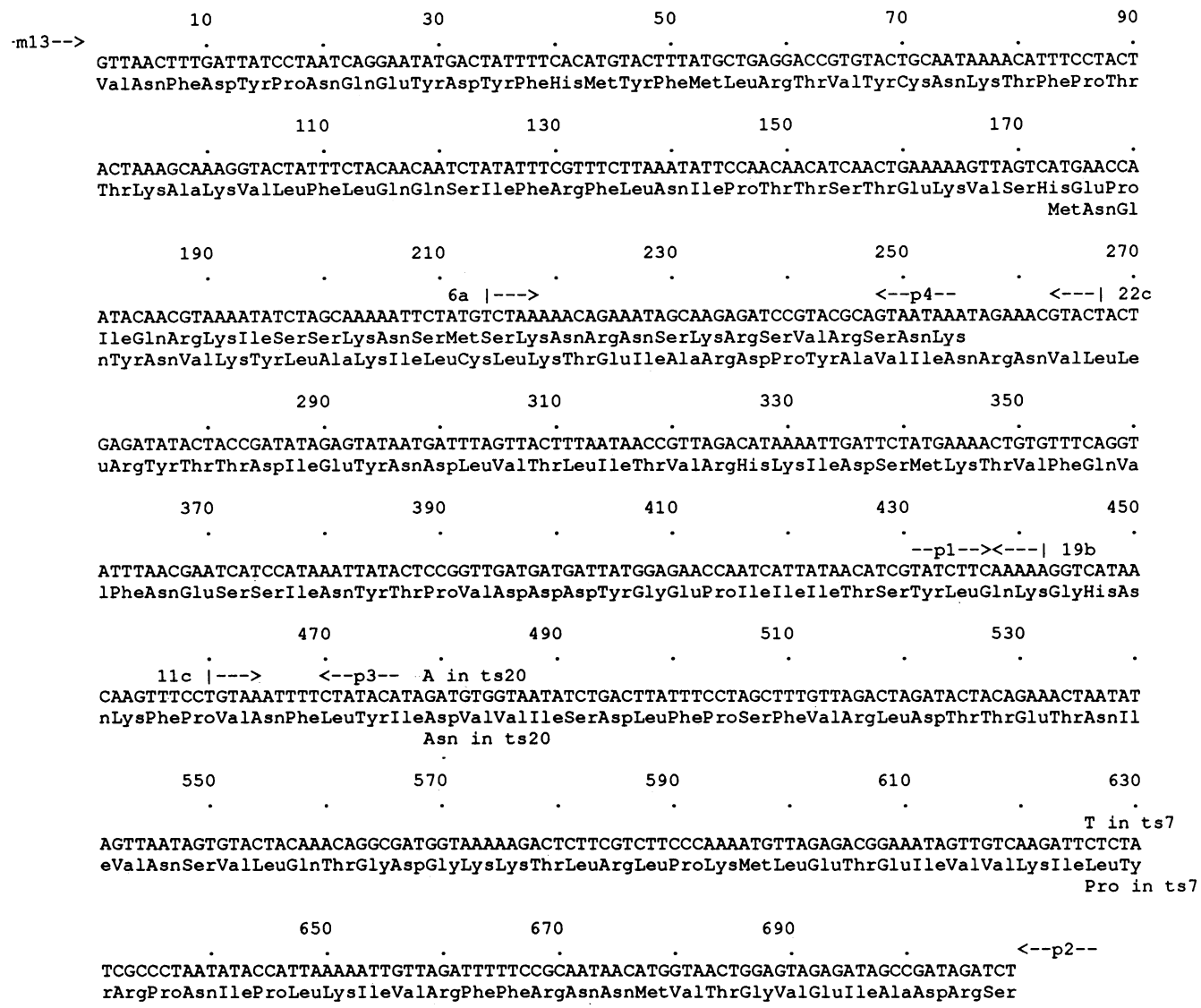

FIG. 5. Sequence of the *Hinc*II-*Bgl*II fragment. The sequence of the wt 709-bp *Hinc*II-*Bgl*II fragment is shown. The translation from nucleotides 1 through 255 represents the carboxy terminus of ORF J3R. The translation from nucleotides 173 through 709 represents the amino terminus of ORF J4R. Positions of primers used in sequencing are indicated (----> or <----), with the arrowhead positioned over the 3′ base of the primers. m13 indicates the M13 universal primer, and p1 through p4 indicate the primers described in Materials and Methods. The 3′ ends of the M13 and p2 primers lie outside of the sequence shown. The endpoints of exonuclease deletion subclones (Materials and Methods and Fig. 4) are indicated (| ---> or <---|), with the vertical line positioned at the deletion endpoint and the arrowhead pointing in the direction of DNA included in the clone. The nucleotide and amino acid changes in *ts*7 and *ts*20 are also shown.

ase and *ts*51, *ts*53, and *ts*65 are members of the same complementation group, we conclude that these three temperature-sensitive mutations reside in the 147-kDa subunit of the viral RNA polymerase.

Mutations in the 22-kDa subunit of the vaccinia virus RNA polymerase. The temperature-sensitive lesions of *ts*7 and *ts*20 had previously been mapped to the *Hin*dIII J fragment (29). Sequence analysis of this region had shown the presence of six different ORFs (Fig. 1). In order to map these mutations to a single ORF, subclones constructed from this fragment (Fig. 1) were used in a marker rescue experiment. The results are shown in Fig. 3 and Table 2. *ts*7 and *ts*20 were rescued by *Hin*dIII-J and by subclones J6 and J7. The subclones J4, J5, and J8 did not rescue *ts*7 and *ts*20. Thus, the two *ts* mutations must lie within a 709-bp *Hinc*II-*Bgl*II fragment in Hind III-J (Fig. 1). The *Hinc*II-*Bgl*II fragment cloned from the wt virus rescued both *ts*7 and *ts*20 (Fig. 3 and Table 2). Sequence data and transcriptional analysis of the *Hinc*II-*Bgl*II region within the *Hin*dIII J fragment showed the presence of two overlapping transcripts and two overlapping ORFs (see Fig. 1, 5, and 7). An ORF for a 41-kDa polypeptide of unknown function ends with a TAG 256 bp downstream from the *Hinc*II site. An ORF for a 22-kDa polypeptide, identified to be one of the small subunits of the viral RNA polymerase, starts 173 bp downstream from the *Hinc*II site.

To refine the map positions for *ts*7 and *ts*20, marker rescue was done by using deletion subclones of the 709-bp *Hinc*II-*Bgl*II fragment. The map positions of the subclones are shown in Fig. 4, and the marker rescue results for *ts*20 are

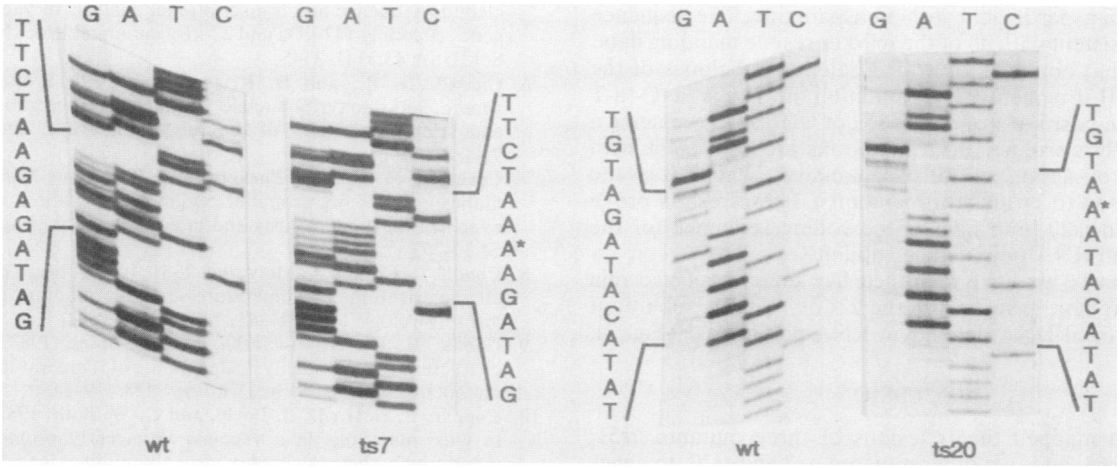

FIG. 6. DNA sequence of *ts*7 and *ts*20. Portions of sequencing data showing the mutations in *ts*7 and *ts*20 and the corresponding data from wt DNA are shown. Data for *ts*7 were obtained by using primer p2, and therefore the sequence is the complement of the sequence shown in Fig. 5. Data for *ts*20 were obtained by using primer p1 and therefore has the same sense as that of the sequence shown in Fig. 5. Mutant nucleotides are indicated at the side of the autoradiograms with an asterisk.

shown in Fig. 3 and Table 2. *ts*20 was rescued with clones 11c, 6a, 1a, and 13a but not with clone 19b or 22c. These results position the mutation in *ts*20 within a sequence of 250 bp located immediately to the left of the *Bgl*II site (Fig. 4). This sequence is contained entirely within ORF J4R (Fig. 5), thereby proving that the mutation in *ts*20 is in the small subunit of the viral RNA polymerase. Similar marker rescue experiments with *ts*7 gave ambiguous results (Table 2).

To refine the map positions of *ts*7 and *ts*20 even further, the sequence of the 709-bp *Hinc*II-*Bgl*II region from each mutant was determined and compared with the sequence from wt virus. Sequence information was obtained by cloning a 1.7-kb *Hinc*II fragment from both mutants and from wt virus into M13 vectors in both orientations and by priming synthesis from each template with an appropriate assortment of oligonucleotides, including both the M13 sequencing primers and primers complementary to vaccinia virus sequences. The sequencing strategy and results are shown in Fig. 5, and examples of the sequencing data are shown in Fig. 6. The entire 709-bp *Hinc*II-*Bgl*II region was sequenced from wt virus and the two mutants. The wt sequence was identical to the published sequence (Fig. 5) (5, 24). The two mutants each showed a single base change in the *Hinc*II-*Bgl*II fragment when compared with wt virus DNA. Both mutations are contained in ORF J4R. The mutation in *ts*20 is a G-to-A transition in position 1 of codon 103 of ORF J4R,

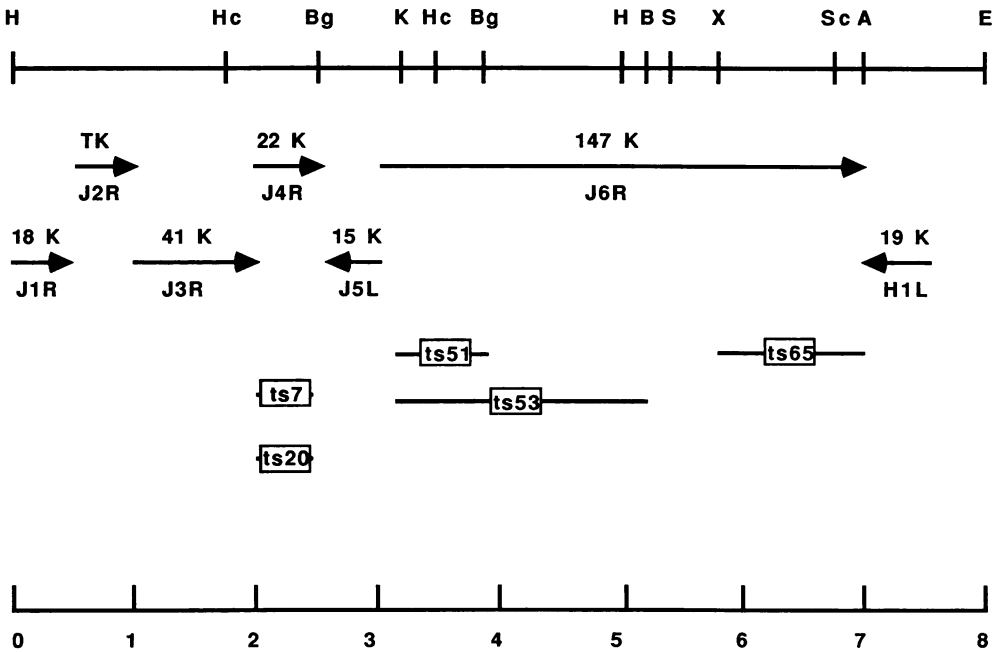

FIG. 7. Diagram of the physical map locations of the *ts* mutations. From the top down, the following are shown. (i) Restriction endonuclease sites within the *Hin*dIII J-H region (see the legend to Fig. 1). (ii) Physical map locations of the ORFs in the *Hin*dIII J-H region. The arrows indicate the direction of translation. (iii) *ts* mutant map positions, deduced as described in the text. (iv) A scale, in kilobases. See the legend to Fig. 1 for other details.

replacing an aspartic acid with an asparagine. The sequence data is consistent with all of the marker rescue mapping data, including that obtained with M13 deletion subclones of the *Hinc*II-*Bgl*II fragment. The mutation in *ts*7 is a C-to-T transition in position 1 of codon 152 of ORF J4R, replacing a leucine with a proline. Both mutations are consistent with the mode of action of nitrosoguanidine, which was the mutagen used to produce the mutants. These results prove that *ts*7 and *ts*20 both map in the coding sequence for the 22-kDa viral RNA polymerase subunit.

In summary, we have identified five mutants of vaccinia virus which have *ts* lesions in the 22- or 147-kDa subunit of the virus-coded DNA-dependent RNA polymerase (Fig. 7).

DISCUSSION

We have mapped the *ts* lesions of three mutants, *ts*51, *ts*53, and *ts*65, and two other mutants, *ts*7 and *ts*20, to genes that encode the 147- and the 22-kDa subunits, respectively, of the vaccinia virus DNA-dependent RNA polymerase. A detailed phenotypic characterization of these mutants is reported in the accompanying report (14). The phenotypic characterizations of three different mutants, and the *ts* lesions of which map to the 147-kDa subunit of the viral RNA polymerase, have previously been reported (11).

It is probable that additional mutants previously described contain *ts* lesions in other vaccinia virus RNA polymerase subunits. Nine different subunits of the viral RNA polymerase have been mapped to various regions on the vaccinia virus genome (15, 19). A total of 5 of these subunits map to the *Hin*dIII A fragment, where we have mapped 14 *ts* mutants from six different complementation groups (9, 15). The D7R ORF encodes a protein which is similar in molecular weight to an RNA polymerase subunit that has been mapped to the *Hin*dIII D fragment and the *ts* lesion of one of the mutants in our collection, C21, has been mapped to this ORF (15, 22, 27).

The identification of these RNA polymerase mutants provides tools to understand transcription and its regulation in vaccinia virus. Since the vaccinia virus RNA polymerase bears sequence homology and immunologic cross-reactivity with the eucaryotic RNA polymerase II (5, 19), our understanding of the vaccinia virus RNA polymerase may aid in understanding the RNA polymerases of higher eucaryotes.

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