Conservative Transcription of \textit{Helminthosporium victoriae} 190S Virus Double-stranded RNA \textit{in vitro}

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(Accepted 26 January 1989)

SUMMARY

In \textit{in vitro} reactions, the \textit{Helminthosporium victoriae} 190S virus-associated RNA polymerase catalysed the synthesis and release of full-length ssRNA transcripts of genomic dsRNA. The transcriptase activity, which was dependent on virus concentration, required all four nucleoside triphosphates and magnesium ions. In reaction mixtures containing [\textsuperscript{3}H]UTP, 99-0\% to 99-5\% of the incorporated label was in ssRNA. Hybridization analysis and \textit{in vitro} translation of the reaction products showed that transcription was asymmetric and that the product of transcription was the message strand. In rabbit reticulocyte lysates, the \textit{in vitro} transcript directed the synthesis of the capsid polypeptide p88. In transcription reactions containing [\textsuperscript{3}H]UTP, no incorporated label was detected in genomic dsRNA during the time it took for the ssRNA transcript to reach full length. These results support the idea that transcription of dsRNA of this virus, like that of dsRNA of other members of the family \textit{Totiviridae}, is conservative.

INTRODUCTION

The 190S virus of \textit{Helminthosporium victoriae}, a member of the family \textit{Totiviridae} (Brown, 1986), has isometric particles 40 nm in diameter and possesses a single species of 4.5 kbp dsRNA (Sanderlin & Ghabrial, 1978). The virus capsid contains two major polypeptides of \textit{M}, 88000 (88K) and 83K (p88 and p83) and a minor polypeptide p78. Peptide mapping by limited proteolysis and selective chemical cleavage at tryptophan residues has shown that p78 and p83 are closely related to p88 (Ghabrial \textit{et al.}, 1987). Because the origin of p78/p83 could not be explained by proteolysis of p88 during virus preparation and storage, it has been suggested that the smaller polypeptides may represent post-translational cleavage and/or modification products of p88 (Ghabrial, 1988; Ghabrial \textit{et al.}, 1987). In rabbit reticulocyte lysates, denatured dsRNA has been shown to direct the synthesis of p88 but no translation products comparable in size to p83 or p78 were detected. Thus, the capsid protein of the 190S virus is encoded by a single gene (Ghabrial \textit{et al.}, 1987).

The dsRNA genome of the 190S virus, like that of other members of the family \textit{Totiviridae}, is at least dicistronic (Buck, 1986). Because the capsid polypeptide p88 is the principal translation product produced by denatured dsRNA (Ghabrial \textit{et al.}, 1987), the gene for the capsid protein is presumed to map near the 5' terminus of the positive strand of dsRNA. The cistron nearer to the 3' terminus is proposed to encode the RNA-dependent RNA polymerase (Ghabrial, 1988). This is supported by the recent finding that the 1135 nucleotides at the 3' terminus of the dsRNA of the \textit{Totiviridae} type member \textit{Saccharomyces cerevisiae} virus LA (ScV-LA) codes for amino acid sequences that share four highly conserved regions with RNA-dependent RNA polymerases of several eukaryotic viruses (Pietras \textit{et al.}, 1988).

The \textit{in vitro} RNA polymerase activity associated with virions of several isometric dsRNA mycoviruses has been shown to catalyse the synthesis of full-length transcripts of genomic dsRNA (Buck, 1986). Present evidence strongly indicates that transcription and replication of \textit{S. cerevisiae} ScV-LA dsRNA occurs conservatively both \textit{in vivo} and \textit{in vitro}, in much the same way as has been established for reoviruses (Newman \textit{et al.}, 1981; Sclafani & Fangman, 1984;
Joklik, 1985; Fujimura et al., 1986; Nemeroff & Bruenn, 1986). In contrast, transcription of dsRNA in fungal viruses with bipartite genomes, those belonging to the family Partitiviridae, occurs by a semi-conservative mechanism (Buck, 1978, 1986; Buck et al., 1981). It was thus of interest to examine the mode of transcription in H. victoriae 190S virus, a member of the family Totiviridae, that infects a filamentous fungus. Preliminary accounts of portions of this study have been reported elsewhere (Ghabrial & Havens, 1986, 1987; Ghabrial, 1988).

METHODS

Virus source and purification. H. victoriae isolate B-1 (ATCC 42019) was used as a source of the 190S virus. Ten 14-day-old stationary cultures of isolate B-1 grown on potato dextrose broth supplemented with 0.5% (w/v) yeast extract were processed for virus purification as described by Sanderlin & Ghabrial (1978) except that mycelium was homogenized in 0.1 M-sodium phosphate buffer pH 7.0, containing 0.2 M-KCl and 0.5% 2-mercaptoethanol.

RNA polymerase assay. The virion-associated RNA polymerase activity was tested in samples of the 190S virus purified by sucrose density gradient centrifugation. Unless otherwise stated, the standard reaction mixtures contained 50 mM-Tris–HCl pH 8.0, 5 mM-MgCl₂, 20 mM-NaCl, 5 mM-KCl, 10 mM-2-mercaptoethanol, 0.1 mM-EDTA, 0.5 mM-ATP, 0.5 mM-GTP, 0.5 mM-CTP, 0.02 mM-[³H]UTP (2 Ci/mmol), bentonite (800 μg/ml) and virus (250 μg/ml). In some experiments, 0.02 mM-UTP and 10 μCi [³²P]UTP (600 Ci/mmol) were used in place of [³H]UTP. The reaction mixtures were incubated at 30 °C. To measure the incorporation of [³H]UTP into acid-insoluble products, 10 μl aliquots of the reaction mixtures were incubated with 50 μg yeast tRNA and 2 ml 10% (w/v) trichloroacetic acid (TCA) at 0 °C for 15 min. The resulting precipitate was collected on glass fibre filters (Whatman GF/C) and washed four times each with 5 ml 10% TCA and twice with 5 ml 95% ethanol. Filters were dried and radioactivity was measured by liquid scintillation.

Characterization of reaction products. The RNA products of standard polymerase reaction mixtures containing either [³H]UTP or [³²P]UTP were isolated by phenol/SDS extraction as follows. The reaction mixtures were made to 25% SDS and heated to 60 °C for 15 min. The mixtures were then extracted with phenol and the RNA was ethanol-precipitated. Unless otherwise indicated, the RNA products of a 2 to 4 h polymerase reaction were used. To determine the size and nature of the radiolabelled RNA, the reaction products were analysed by electrophoresis on 4% gels containing 8 M-urea (Buck & Ratti, 1977).

Separation of ssRNA and dsRNA. RNA extracted from polymerase reaction mixtures was dissolved in TE buffer (10 mM-Tris–HCl pH 7.4, 1 mM-EDTA), made to 2 M with LiCl, and incubated overnight at 0 °C. The mixture was centrifuged in an A-100 rotor in a Beckman airfuge at 200 kPa for 30 rain or in an Eppendorf centrifuge for 30 rain at 15600 g. The pellets were dissolved in STE buffer (50 mM-Tris–HCl, 0.1 M-NaCl, 1 mM-EDTA) pH 7.0, repurified from 70% ethanol, and the RNA was recovered by centrifugation. The pellets were dissolved in 100 to 200 μl STE containing 15% ethanol (STE/15) and layered on CF-11 (or Cellex N-1) cellulose mini-columns (0.5 ml plastic tube containing 25 to 50 mg CF-11 cellulose previously equilibrated in STE/15 and packed over siliconized glass wool). The eluate was recovered by piercing a small hole in the bottom of the tubes, nesting the tube inside a larger, 1.5 ml centrifuge tube, and centrifuging for 10 s in an Eppendorf centrifuge. The column was washed once with 200 μl STE/15, then with 200 μl STE. The original eluate and the STE/15 wash were combined and applied to a second spin column. The eluate and the STE/15 wash from the second column were combined and the ssRNA was recovered by ethanol precipitation.

RNase treatments. The labelled RNA products from a standard RNA polymerase reaction mixture, from which bentonite was omitted, were incubated with RNase A (10 μg/ml) in low or high salt concentrations at 37 °C for 30 min. High salt concentrations (0.5 and 1 M) were obtained by adding appropriate volumes of 5 M-NaCl to the reaction mixtures. Resistance or sensitivity to RNase was assessed on the basis of the amount of radioactivity that remained acid-insoluble following treatment. When monitored by agarose gel electrophoresis and ethidium bromide staining or autoradiography. In some experiments, the reaction mixtures were phenol-extracted and the RNA was concentrated by ethanol precipitation before analysis by agarose gel electrophoresis.
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In vitro translation. Cell-free protein synthesis was performed at 30 °C for 60 min in mRNA-dependent rabbit reticulocyte lysates. A standard reaction mixture of 50 μl containing 50 μCi [35S]methionine was mixed with 1 to 4 μg of denatured dsRNA, undenatured dsRNA, or ssRNA in vitro transcripts. Labelled translation products were analysed by SDS–PAGE and autoradiography.

Immunoprecipitation. Immunoprecipitation of translation products was performed according to Franssen et al. (1982) using anti-190S virus antiserum.

Peptide mapping. Translation products identified by autoradiography were cut from dried gels and subjected to selective chemical cleavage using N-chlorosuccinimide (NCS)/urea as described by Lischwe & Ochs (1982). The NCS-generated peptides were analysed by SDS–PAGE and the gels were silver-stained using the method of Wray et al. (1981). Following destaining and drying, the gels were autoradiographed.

RESULTS

Purification of the 190S virus

The diseased isolate B-1 of H. victoriae is known to contain two isometric dsRNA viruses, designated according to the sedimentation of their particles as the 190S and 145S viruses (Sanderlin & Ghabrial, 1978). Purified virus preparations obtained from subcultures of this isolate contained considerably larger amounts of the 190S virus than of the 145S virus. When examined by electron microscopy, gradient fractions representing the 190S peak contained isometric particles 40 nm in diameter. Polyacrylamide gel electrophoresis of RNA isolated from gradient-purified 190S virus revealed a single species of dsRNA of Mr approx. 3.0 × 10⁶ (4.5 kbp).

Characteristics of RNA polymerase activity of the 190S virus

When gradient-purified 190S virus was incubated in a standard RNA polymerase reaction mixture containing [3H]UTP, radioactivity was incorporated into TCA-insoluble products. The polymerase activity was approximately proportional to virus concentration in the range of 50 to 250 μg/ml (Fig. 1). At a concentration of 250 μg virus per ml, the polymerase activity was linear for the first 2 h; then the reaction slowed down but did not level off even after 4 h (Fig. 1). The polymerase activity was insensitive to actinomycin D and rifampin but required all four nucleoside triphosphates (Table 1). Although bentonite had little or no effect on [3H]UTP incorporation (Table 1), it or RNasin (1 unit/μl of reaction mixture) was used in all assays as a precautionary measure.

The polymerase reaction required Mg²⁺ (Table 1) with optimum activity at 5 mM. The optimum pH for the polymerase reaction was 8.4, as determined by varying the pH of the reaction mixture between 7.0 and 9.0 using Tris–HCl buffer.

Analysis of reaction products

Nature of labelled RNA products

Several approaches were used to determine the strandedness of the labelled RNA isolated from RNA polymerase mixtures containing [3H]UTP. Both selective precipitation of ssRNA from 2 M-LiCl and chromatography on CF-11 cellulose columns indicated that the radioactive label was predominantly incorporated into ssRNA (86 to 95% of the total acid-insoluble radioactivity). It was apparent, however, that one cycle of either procedure was not adequate to obtain accurate estimates of [3H]UTP incorporation into ssRNA. For example, when the 2 M-LiCl supernatant fraction, which is enriched in dsRNA, was chromatographed on a CF-11 cellulose column, 98.5% of the TCA-insoluble radioactivity was eluted with STE/15; i.e. incorporation of radioactivity was in ssRNA. Thus, to remove contaminating dsRNA from ssRNA preparations and to obtain more accurate values for [3H]UTP incorporation into ssRNA, the LiCl-spin column procedure detailed earlier was followed. At least one or two cycles of differential precipitation in 2 M-LiCl followed by two cycles of chromatography on Cellex N-1 spin columns were needed to remove detectable dsRNA from polymerase reaction mixtures. Monitoring the TCA-insoluble radioactivity in the various fractions showed that 99.0% to 99.5% of the incorporated label was in ssRNA. These results which suggest that there is very little or no incorporation of label into dsRNA were supported by the finding that no...
Fig. 1. Dependence of the 190S virus-associated RNA polymerase reaction on virus concentration. Standard RNA polymerase reaction mixtures (200 µl) containing 50 (■), 125 (■) or 250 (▲) µg/ml virus were used; 10 µl samples were withdrawn at the times shown and assayed for TCA-insoluble radioactivity. Values for radioactivity were those obtained with 10 µl samples.

Table 1. Characteristics of the RNA polymerase activity associated with H. victoriae 190S virus

<table>
<thead>
<tr>
<th>Reaction mixture</th>
<th>Activity (% of standard)</th>
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<tbody>
<tr>
<td>Complete</td>
<td>100</td>
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<tr>
<td>+ Actinomycin D (125 µg/ml)</td>
<td>96</td>
</tr>
<tr>
<td>+ Rifampin (10 µg/ml)</td>
<td>91</td>
</tr>
<tr>
<td>- Bentonite</td>
<td>92</td>
</tr>
<tr>
<td>- Mg²⁺</td>
<td>4</td>
</tr>
<tr>
<td>- Virus</td>
<td>3</td>
</tr>
<tr>
<td>- ATP, CTP, GTP</td>
<td>4</td>
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<tr>
<td>- Bentonite, - NTP</td>
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radioactivity could be detected in gel slices containing dsRNA after overnight digestion in Protosol (New England Nuclear).

Digestion of the polymerase reaction product (14266 c.p.m.) with RNase in 0·5 M-NaCl, 0·3 M-NaCl, or in the absence of added salt confirmed its single-stranded nature as it was almost completely degraded by RNase under both high salt (879 c.p.m. and 424 c.p.m. acid-insoluble radioactivity for the 0·5 M- and 0·3 M-NaCl treatments respectively) and low salt conditions (313 c.p.m. acid-insoluble radioactivity). When monitored by agarose gel electrophoresis and ethidium bromide staining, the 190S virus dsRNA was found to be completely resistant to RNase under the high salt conditions of the assay whereas ssRNA standards were completely degraded (data not shown).

Size of the ssRNA transcript

Electrophoretic analysis of labelled RNA, isolated from RNA polymerase reaction mixtures containing [α-32P]UTP, revealed a major band that comigrated with denatured dsRNA (Fig. 2, lane 6). The 190S virus dsRNA was denatured by incubation in 90% dimethyl sulphoxide for 15 min at 50 °C and used as an ssRNA marker (4·5 kb) along with SBMV and PSV RNAs (Fig. 2). In addition to the full-length ssRNA transcript, several bands of shorter length, generally occurring in minor amounts, were detected by autoradiography. It is not clear whether these bands represent degradation products, partial transcripts, or pause products in the synthesis of full-length transcripts. All labelled products, nevertheless, have been shown to be single-
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Fig. 2. Electrophoresis in 4% polyacrylamide gel containing 8 M-urea of RNA polymerase product extracted from a 4 h reaction mixture containing [α-32P]UTP. Ethidium bromide-stained gel (lane 5), and autoradiogram (lane 6); arrowhead (lane 5) indicates the position of the full-length ssRNA transcript. Controls (stained with ethidium bromide) were: lane 1, SBMV RNA (4.1 kb); lane 2, PSV RNA 1 to 4 (3.4, 3.0, 2.2 and 1.0 kb, respectively); lane 3, denatured 190S virus dsRNA (4.5 kbp); lane 4, undenatured 190S virus dsRNA.

Fig. 3. Hybridization of RNA polymerase product to *H. victoriae* 190S virus dsRNA. [3H]UTP-labelled ssRNA (30000 c.p.m.) was hybridized with increasing amounts of unlabelled virus dsRNA. The extent of hybridization was expressed as the percentage of labelled ssRNA rendered RNase-resistant under high salt conditions.

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stranded since no label could be detected by autoradiography when the reaction products were first incubated with RNase in either high or low salt conditions and then subjected to electrophoresis (data not shown). Furthermore, no incorporation of radioactive label into the dsRNA band could be detected when the ethidium bromide-stained gel was autoradiographed (Fig. 2, lanes 5 and 6).

**Hybridization analysis**

When 3H-labelled ssRNA was incubated under annealing conditions with increasing amounts of denatured unlabelled dsRNA, an increasing proportion, up to 97%, of the ssRNA products hybridized (Fig. 3). Negligible hybridization was detected when tritiated ssRNA was incubated under annealing conditions without denatured genomic dsRNA (Fig. 3). These results and the finding that, in a cell-free system, the *in vitro* transcript acts as mRNA for the synthesis of the capsid polypeptide p88 (see section on translation of *in vitro* transcript), suggest that only one strand of the parental dsRNA is being transcribed and that the product of *in vitro* transcription is the plus 'message' strand.

**Release of ssRNA transcripts**

In a reaction time-course experiment, aliquots of total polymerase reaction mixtures containing [α-32P]UTP were analysed directly by agarose gel electrophoresis in 1 to 1.5% agarose gels. Full-length ssRNA transcripts were first detected after 60 min of incubation (Fig. 4). The mobility of SBMV RNA (4.1 kb) was used as a guide in locating the position of full-length ssRNA transcript (6.5 kb) of the 190S virus dsRNA; this would be expected to migrate slightly more slowly on 1% agarose gels than SBMV RNA (Fig. 4). Treatment of reaction mixtures with
RNase under high salt conditions before electrophoresis resulted in complete degradation of labelled products which suggested that the ssRNA transcripts had been released from the particles. The finding that the small amount of free dsRNA, as visualized by ethidium bromide staining (Fig. 4), remained largely unchanged during 4 h of incubation (when compared to a virus sample of similar amount that had not been incubated under transcription conditions; Fig. 4, lane 11) indicates that breakdown of transcribing particles did not contribute significantly to the release of ssRNA transcripts.

In repeated time-course experiments in which reaction mixtures were analysed by agarose gel electrophoresis either directly or following phenol extraction of reaction products (Fig. 5), we were able to detect full-length ssRNA between 30 to 60 min of incubation. Assuming that the polymerase reaction starts at zero time in all active particles, the rate of nucleotide incorporation into ssRNA in vitro is about 75 to 150 nucleotides/min (the size of full-length ssRNA is 4500 nucleotides). The percentage of virus particles active in transcription in the RNA polymerase reaction was calculated as follows. Samples of the same virus preparation used in the time-course experiment described in Fig. 4 were assayed in a standard RNA polymerase reaction mixture containing [3H]UTP. The final specific activity of UTP in the reaction mixture was 2.0 Ci/mmol. Assuming UMP represents 0-25 of the ssRNA synthesized and has a residue Mr of 330, the specific activity of ssRNA was calculated as 1.3 × 10^6 c.p.m./μg (the counting efficiency of 3H was determined as 40%). In a 100 μl standard assay with 10 μg virus (equivalent to 2 μg dsRNA), the total radioactivity incorporated after 1 h was 2.56 × 10^6 c.p.m. This is equivalent to 0.2 μg ssRNA. Thus, 0.1 molecule of ssRNA was synthesized per h per one molecule of dsRNA. Based on a nucleotide chain elongation rate of 75 to 150 nucleotides/min, one can calculate that 10 to 20% of the virus particles were active in ssRNA synthesis. Using a similar approach, Fujimura et al. (1986) calculated that 30% of the ScV-LA particles were enzymically active in a polymerase reaction.

**Translation of in vitro transcript**

In a rabbit reticulocyte lysate cell-free system, the in vitro transcripts from a 4 h standard polymerase mixture have been found to direct the synthesis of a product that comigrates with authentic capsid polypeptide p88 (Fig. 6, lane 2), reacts with an antiserum to the 190S virus (Fig. 6, lane 7), and produces peptide maps after selective chemical cleavage at tryptophan residues identical to those of p88 translated from denatured 190S virus dsRNA (Fig. 7). As previously shown (Ghabrial et al., 1987), denatured 190S virus dsRNA can be translated to produce a single major polypeptide identical to the capsid polypeptide p88 (Fig. 6, lanes 2 and 3). No translation
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Fig. 5. Autoradiogram from a time course experiment of the RNA polymerase activity in virions of the 190S virus. Samples were taken at different times (10, 30, 40, 60, 90, 120 and 150 min, lanes 1 to 7, respectively) from the reaction mixture, phenol-extracted, and analysed by agarose gel electrophoresis on 1\% agarose gels. O, origin.

Fig. 6. Autoradiography of translation products of denatured dsRNA, undenatured dsRNA and ssRNA transcript (lanes 2, 4 and 6, respectively) and immunoprecipitates of products of the same three RNAs (lanes 3, 5 and 7, respectively). Positions of \textit{M} standards (×10^{-3}) were marked with radioactive ink (lane 1).

products were detected when undenatured dsRNA was used (Fig. 6, lanes 4 and 5) or in control reactions where no exogenous mRNA was added (data not shown).

In addition to p88, the translation products of the \textit{in vitro} transcripts included smaller polypeptides which were also immunoprecipitated using an antiserum to the 190S virus (Fig. 6, lane 7). To investigate the origin of these secondary translation products further, RNA from polymerase reaction mixtures was fractionated by centrifugation on linear log-density gradients (Sanderlin & Ghabrial, 1978) and the RNA fractions were translated in rabbit reticulocyte lysates. The gradient fractions were also monitored by u.v. absorbance at 254 nm, counting by liquid scintillation, and polyacrylamide gel electrophoresis in 8\% urea followed by fluorography. The results showed that full-length ssRNA, as revealed by PAGE and fluorography (Fig. 8a, lane 1), was resolved as a single peak sedimenting at a faster rate than genomic dsRNA (data not shown). Translation of gradient-purified full-length ssRNA gave rise mainly to p88, and lesser amounts of smaller polypeptides (Fig. 8b, lane 3). On the other hand, translation of \textit{in vitro} transcripts that had not been subjected to gradient centrifugation produced relatively large amounts of the smaller polypeptides (Fig. 8b, lane 5). Translation of gradient fractions containing more slowly sedimenting molecules with shorter than full-length ssRNA (Fig. 8a, lanes 2 and 3) gave rise only to the smaller polypeptides. Thus, the appearance and amounts of the secondary translation products appear to correlate with the presence in the transcription mixture of RNA molecules shorter than full-length transcripts.

A polypeptide with an \textit{M}\textsubscript{r} of approx. 42K (Fig. 8b, arrow) was a major product of the \textit{in vitro} translation system. Because of its weak reactivity to the anti-190S virus serum (Fig. 6, lane 7), it was of interest to determine its relationship to p88. For this purpose, bands corresponding to the
Fig. 7. Autoradiogram (lanes 1 to 3) and silver-stained polyacrylamide gels (lanes 4 to 7) of peptides generated by NCS. Positions of M_r standards (×10^-3) are indicated to the right. Lane 1, NCS-treated translation product p88 of \textit{in vitro} transcript; lane 2, NCS-treated translation product p88 of denatured genomic dsRNA; lane 3, positions of NCS-generated peptides of capsid p88, viewed by silver staining, and marked by radioactive ink (same as lane 4); lane 4, NCS-cleaved p88; lane 5, NCS-cleaved p78; lane 6, untreated p78; lane 7, untreated p88.

Fig. 8. (a) PAGE of RNA polymerase product extracted from a 4 h reaction mixture containing [3H]UTP and fractionated by linear-log density gradient centrifugation (Sanderlin & Ghabrial, 1978). Gradient fractions were analysed by PAGE on 4% polyacrylamide gels containing 8 M-urea followed by fluorography. Lane 1, gradient fraction containing full-length ssRNA as indicated by co-electrophoresis with denatured dsRNA; lanes 2 and 3, slower sedimenting gradient fractions containing short ssRNA molecules. (b) \textit{In vitro} translation products of full-length ssRNA (gradient fraction shown in Fig. 8a lane 1) and shorter RNA molecules (gradient fractions shown in Fig. 8a lanes 2 and 3) are shown in lanes 3 and 4, respectively. Translation products of the polymerase products before density gradient centrifugation are shown in lane 5. No exogenous RNA was added to the translation mixture in lane 1. The positions of the M_r standards (×10^-3) are marked with radioactive ink (lane 2).
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Fig. 9. Autoradiogram showing comparative peptide mapping of translation products p88 and the 42K polypeptide indicated by arrowhead in Fig. 8(b). The peptides were generated by NCS treatments of bands cut from SDS gels; lane 1 and 2, NCS-treated 42K polypeptide; lane 3, NCS-generated peptides of translation product p88; lane 4, positions of bands in silver-stained track of NCS products of capsid polypeptide p88. Positions of Mr markers (× 10^{-3}) are indicated to the right.

two polypeptides were cut from dried gels, treated with NCS, and the generated peptides were analysed by SDS-PAGE and autoradiography. The results shown in Fig. 9 indicate that the 42K polypeptide is closely related to p88 since their peptide profiles are identical. As would be expected, peptides larger than 42K (Fig. 7, lanes 1 and 2) were not detected among the cleavage products of the 42K polypeptide.

DISCUSSION

The in vitro RNA polymerase activity associated with virions of H. victoriae 190S virus has been shown to catalyse the synthesis and release of full-length ssRNA transcripts of virus dsRNA. The transcriptase activity was dependent on virus concentration and required all four nucleoside triphosphates and magnesium ions. The characteristics and requirements of the 190S virus-associated RNA polymerase activity are similar to those reported for other isometric dsRNA mycoviruses (Herring & Bevan, 1977; Buck, 1979; Ratti & Buck, 1979; Welsh et al., 1980; Buck et al., 1981; Ben-Tzvi et al., 1984).

The finding that transcription in the presence of [3H]UTP leads to no or extremely low levels (0.5 to 1.0%) of label incorporation in genomic dsRNA suggests that transcription occurs conservatively such that the integrity of the parental dsRNA remains intact and the newly synthesized positive strand is released from the particle. This is in contrast to a semi-conservative mechanism in which the newly synthesized ssRNA displaces the parental positive strand and the latter is then released from the virion. The possibility that only a very small proportion (0.5 to 1.0%) of the particles might be active in transcription and rapidly synthesizing positive strands semi-conservatively could be ruled out for two reasons. First, no label was incorporated in dsRNA during the time it took for the newly synthesized ssRNA to reach full length (Fig. 4 and 5). If a minute proportion of particles were rapidly synthesizing transcripts semi-conservatively then label would be expected to be incorporated in dsRNA before the formation of full-length ssRNA. Secondly, we have estimated that 10 to 20% of the 190S virions, purified from 14-day-old stationary cultures, were active in transcription.
It is now well accepted that in vitro transcription and replication of the yeast virus ScV-L_A, the type species of the family Totiviridae, occur conservatively (Fujimura et al., 1986, 1988). Conservative transcription has also been reported or suggested by published results for two other members of the Totiviridae infecting yeast or fungi with yeast-like growth, namely the yeast virus ScV-L_Bc (Esteban & Wickner, 1986) and Ustilago maydis virus P6-H1 (Ben-Tzvi et al., 1984). The results of the present study strongly favour a conservative mode of transcription for H. victoriae 190S virus, a member of the Totiviridae that infects a filamentous fungus. Future studies will probably prove conservative transcription and replication to be the rule for viruses in this family.

The in vitro translation studies have shown that the full-length ssRNA transcript serves as mRNA for the synthesis of the capsid polypeptide p88. A number of smaller polypeptides serologically related to p88 were also synthesized (Fig. 6 and 8). The extent to which these smaller peptides were produced appeared to be influenced by the presence in the RNA transcript preparation of ssRNA shorter than the full-length transcripts. The origin of these smaller RNA molecules, which generally occur in minor amounts, is not clear. They may represent partial transcripts, degradation products, or pause products in the synthesis of full-length ssRNA transcripts. Alternatively, initiation of transcription may be asynchronous giving rise to populations of RNA molecules at different stages of chain length. The finding that the shorter RNA molecules have message activity for the synthesis of polypeptides related to p88 (Fig. 8 and 9) suggests they may have similar nucleotide sequences at their 5' termini. This needs to be verified by using probes specific for the 5' terminal sequences of the positive strand of 190S dsRNA. Brennen et al. (1981) reported the occurrence of pause products, 300 to 600 nucleotides in length, in preparations of in vitro transcript of the yeast ScV-L_A dsRNA. These 5' truncated transcription products constituted 5% of the RNA synthesized in vitro.

In vitro dsRNA synthesis has been demonstrated with the yeast virus ScV-L_A in virions less dense than those endowed with the ability to transcribe dsRNA into ssRNA (Fujimura et al., 1986). These replicative particles which catalyse the synthesis of negative strand RNA on positive strand templates have been isolated from logarithmically growing yeast cells. The ssRNA-synthesizing 'transcriptional' particles, on the other hand, are isolated from stationary yeast cultures. Thus, by analogy to the yeast virus ScV-L_A, it is not surprising that dsRNA synthesis could not be demonstrated in virions of the 190S virus isolated from 14 day stationary cultures. In future studies, dsRNA-synthesizing particles will be sought in younger shake cultures of H. victoriae.

Journal paper 88-11-254 of the Kentucky Agricultural Experiment Station.

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(Received 14 October 1988)