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TRANSCRIPTASE ACTIVITY ASSOCIATED WITH A TYPE 2 DOUBLE-STRANDED RNA MYCOVIRUS

R.M. McGinty*, K.W. Buck* and C.J. Rawlinson†

*Department of Biochemistry
Imperial College of Science and Technology
London SW7 2AZ UK

†Plant Pathology Department
Rothamsted Experimental Station
Harpenden Herts AL5 2JQ UK

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SUMMARY

It is shown that the virion-associated RNA polymerase of Phialophora virus A, a type 2 double-stranded RNA mycovirus with a genome consisting of three RNA species, is a transcriptase. Synthesis of single stranded RNA in vitro continues for a least 24 hours and after this time two full length transcripts are produced, on average, per dsRNA molecule, i.e.re-initiation of transcription occurs in this in vitro system. Analysis of the products by polyacrylamide gel electrophoresis indicated that the efficiences of transcription of all three double-stranded RNA species were similar. The transcriptase activity of Phialophora virus A differs from the replicase activity of Penicillium stoloniferum virus S, the only other type 2 double-stranded RNA mycovirus whose RNA polymerase has been characterised.

INTRODUCTION

Isometric viruses with genomes of dsRNA occur commonly in fungi (1). There are two basic types; type 1 viruses have an undivided genome of polycistronic dsRNA, whereas type 2 viruses have a genome which is divided into two or more segments of monocistronic dsRNA. Viruses of either type may carry, in some fungal strains, additional segments of satellite or defective dsRNAs which are not essential for virus replication (2). Virion-associated RNA polymerases have been found in viruses of both types (3), although

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Abbreviations: dsRNA - double-stranded RNA; ssRNA - single-stranded RNA; TCA - trichloroacetic acid.

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only a few of these have been adequately characterised. In Saccharomyces cerevisiae L virus, a type 1 virus, the RNA polymerase in mature virions is a transcriptase which catalyses the synthesis and release of full-length ssRNA copies of one of the strands of the dsRNA genome (4, 5). Transcriptase activity has also been detected in virions of two other mycoviruses with polycistronic dsRNAs, Aspergillus foetidus virus S (6) and Allomyces arbuscula virus (7). In contrast the virion-associated RNA polymerase of Penicillium stoloniferum virus S, a type 2 virus, is a replicase, which catalyses the synthesis in vitro of a new molecule of dsRNA, which remains within the particles, giving rise to diploid virions (8). Since the Penicillium virus replicase was the only RNA polymerase of a type 2 dsRNA mycovirus to have been characterised, it was of interest to know if replicase activity is characteristic of other type 2 dsRNA mycoviruses. Two serologically unrelated type 2 dsRNA mycoviruses (A and B) have been obtained from an isolate of a Phialophora sp. parasitic on barley roots (9). We now report that both of these viruses have virion-associated RNA polymerase activity and that the virus A enzyme is a transcriptase, which catalyses the synthesis of ssRNA copies of each of the three virus dsRNA components. This activity differs from that of Penicillium stoloniferum virus S (8) and this is the first report of transcriptase activity associated with particles of a type 2 dsRNA mycovirus.

METHODS

Preparation of viruses. Viruses A and B from Phialophora sp. (lobed hyphopodia) isolate 2-2 were isolated, purified and separated by the methods described previously (10). Virus preparations were finally dialysed against TNE buffer (0.05M-tris-HCl + 0.15M - NaCl + 0.1mM-EDTA, pH 7.9) and stored at -20°C after addition of an equal volume of glycerol.

RNA polymerase assay. Standard reaction mixtures contained 0.15mM-ATP; 0.15mM - GTP; 0.15mM - CTP; 0.15mM - [^{3}H]UTP (sp. act. 17 to 68mCi/mmol); $MgCl_2$ (2.5mM, virus A; 5.0mM, virus B); actinomycin D (100µg/ml); bentonite (800µg/ml); virus (A_{260} 0.25 to 5); TNE buffer. Incubation was at 30°C. Incorporation of [^{3}H]UMP into acid insoluble material was determined by adding 3ml of 10% (w/v) TCA to 10 to 100µl of chilled reaction mixture

and allowing precipitation to occur at 0°C for 30 minutes. The precipitate was collected on Whatman GF/F filters and washed extensively with 2% TCA and then with ethanol. Filters were dried and radioactivity was determined by liquid scintillation counting in a toluene-based scintillation fluid.

Preparation of RNA. Solutions were made 1% in sodium dodecyl sulphate and then extracted with phenol. The ethanol precipitated RNA was resuspended in the required buffer and then dialysed against the same buffer. For separating ssRNA and dsRNA selective precipitation of ssRNA in 2M-LiCl was used (11).

Analysis of RNA by electrophoresis. Electrophoresis of RNA was carried out in 4% polyacrylamide slab gels in a tris-acetate-EDTA buffer containing 8M-urea (12) at 5 volts/cm for 15h. Gels were either stained with 0.01% aqueous toluidine blue or examined by fluorography (13). Denaturation and glyoxalation of dsRNA prior to electrophoresis, when required, was carried out as described by McMaster & Carmichael (14), except that 60% (v/v), rather than 50% (v/v), dimethyl sulphoxide was employed in the reaction mixture.

RNA hybridization assays. These were carried out by the Method B described by Ratti and Buck (6).

RESULTS AND DISCUSSION

Both viruses A and B catalysed the incorporation of [^{3}H]UMP into TCA-insoluble material in RNA polymerase reaction mixtures. Both reactions were dependent on magnesium ions with optima of 2.5mM and 5mM respectively and no pretreatment of the virions with a proteinase or by heat shock treatment was necessary to activate the polymerase as is the case with certain other dsRNA viruses e.g. reovirus (15). Omission of one of the four nucleoside triphosphates almost completely abolished the reaction in both cases. In the case of virus A [^{3}H]UMP incorporation was linear for at least 30 h whereas the virus B reaction slowed down after a short period of time (Fig.1), probably due to the greater instability of this virus under the reaction conditions.

The products of an 18h RNA polymerase reaction with virus A were isolated by phenol/sodium dodecyl sulphate extraction and examined by electrophoresis in polyacrylamide gels containing 8M-urea. After staining of gels with toluidine blue six clear bands were revealed, three bands corresponding to virus template dsRNAs, mol. wt. 1.29×10^6, 1.22×10^6 and 1.03×10^6 and three bands of newly synthesised RNA of slower mobility.

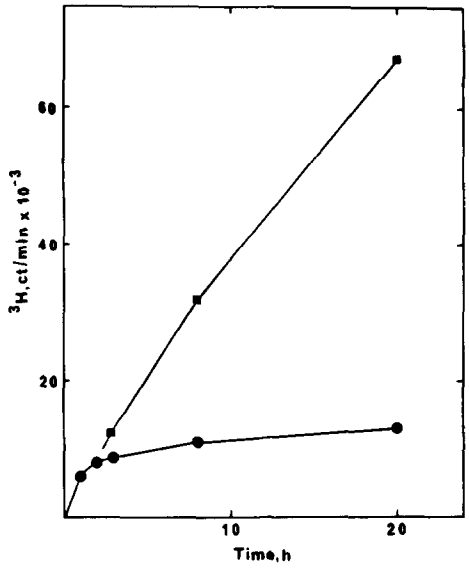

Fig. 1: RNA synthesis catalysed by Phialophora viruses A and B RNA polymerases. Reaction mixtures containing [^{3}H]UTP (specific activity 17.5 mCi/mmol) and virus A or B (2 A_{260} units) were incubated at 30°C as described in the Methods. At the indicated times, 10µl samples were withdrawn and the radioactive material insoluble in cold TCA was determined. ■ virus A; ● virus B.

When the RNA products were incubated with ribonuclease A (0.25µg/ml) for 2h in hybridisation buffer (6), prior to electrophoresis, the three slower moving bands could no longer be observed, but the three bands of virus dsRNA were unchanged. The three newly synthesised RNA components are therefore single-stranded.

In order to prove that the ssRNA products arose by transcription of the virus template dsRNAs, the [^{3}H] ssRNA products of an 18h polymerase reaction were isolated by precipitation with 2M-LiCl and annealed with increasing amounts of denatured unlabelled virus dsRNA. The extent of hybridisation which occurred was measured by the amount of label which remained TCA-insoluble after treatment with ribonuclease A in hybridisation buffer (6). The proportion of products capable of hybridising to the virus template RNA increased with the amount of virus dsRNA up to at least 85% (Fig.2). No self-annealing of the product, in the absence of added denatured virus dsRNA, could be detected. Co-electrophoresis experiments

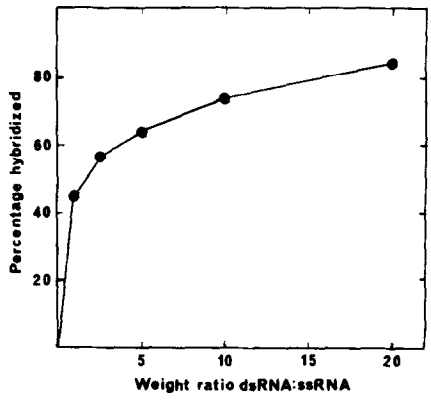

Fig.2: Hybridisation of virus A RNA polymerase product to virus template dsRNA. ^{3}H-labelled ssRNA product (17 000 ct/min) was hybridised with increasing amounts of unlabelled denatured virus dsRNA. Hybridisation is expressed as the percentage of input ssRNA label which becomes resistant to ribonuclease A in high salt buffer.

showed that the three ssRNA products had mobilities identical to those of the three virus dsRNAs, after denaturation and glyoxalation. It was concluded that these products are full length ssRNA transcripts of one of the strands of each of the three virus dsRNA components.

The amount of ssRNA product synthesised after a 24h RNA polymerase reaction was calculated (a) from absorbance at 260nm, assuming A_{260} of 1 is equivalent to 40µg/ml ssRNA and 50µg/ml dsRNA and (b) from [^{3}H]UMP incorporation, assuming a UMP content of 25% for ssRNA. Both methods indicated that a weight of ssRNA approximately equal to that of the template dsRNA had been synthesised, i.e. on average, two rounds of transcription per dsRNA molecule had occurred. This result shows that re-initiation of transcription can occur in the in vitro system. The proportions of individual transcripts, as judged from intensities of bands in toluidine blue stained gels or after fluorography of gels, following electrophoresis of product RNA, were similar to those of the template dsRNA molecules, indicating approximately equivalent efficiencies of transcription of each of the three dsRNA components.

The results have shown that the virion-associated RNA polymerase of the

Phialophora virus A is a transcriptase which catalyses the synthesis of full-length ssRNA copies of one of the strands of each of the three virus dsRNA template molecules. This polymerase differes fundamentally from that of Penicillium stoloniferum virus S which is a replicase. Another difference is that transcription can be initiated in vitro by the Phialophora virus enzyme, whereas the Penicillium virus replicase is unable to initiate the reaction and RNA synthesis takes place only in particles which contain ssRNA primers in addition to template dsRNA(8). These results, which show for the first time transcriptase activity in virions of a type 2 dsRNA mycovirus, suggest sub-division of the type 2 viruses into type 2a, those with virion-associated replicase activity and type 2b, those with virion-associated transcriptase activity.

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