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Replication of double-stranded RNA in particles of Penicillium stoloniferum virus S

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ABSTRACT

RNA polymerase activity was assayed in different particle classes of Penicillium stoloniferum virus S. RNA polymerase activity was found to be associated with H particles, which contain double-stranded RNA and single-stranded RNA, but not with L particles, which contain only double-stranded RNA and not with M particles, which contain only single-stranded RNA. In H particles the reaction occurred with the formation of one new molecule of double-stranded RNA (or two complementary single strands of RNA) per virus particle and the production of product particles (P particles), which contained two molecules of double-stranded RNA (or its equivalent). This RNA polymerase is therefore a replicase, which catalyses the synthesis of the two complementary strands of double-stranded RNA in a single virus particle. This is the first report of this type of RNA polymerase system.

INTRODUCTION

RNA polymerase activity has been shown to be associated with virions (or cores derived from virions by proteinase action) of double-stranded RNA viruses of animals and higher plants, such as reovirus, cytoplasmic polyhedrosis virus and wound tumour virus[1]. In these cases the enzymes function as transcriptases and the multiple copies of virus messenger RNA molecules produced are released from the particles. In addition sub-viral particles, isolated from reovirus infected cells, were found to contain a different RNA polymerase activity, synthesising double-stranded RNA on a single-stranded RNA template; in this case the RNA product remained associated with the sub-viral particles, which are believed to be precursors of mature virions[2].

Recently RNA polymerase activity has been detected in particles of double-stranded RNA viruses from a number of fungi (Penicillium stoloniferum[3], Penicillium chrysogenum[4] and Aspergillus foetidus[5]). In the case of P. stoloniferum viruses the product of reaction was double-stranded RNA of the same molecular weight as virus genomic RNA, and remained within the particles[6]. Because of the limited amount of RNA synthesis obtained, it was postulated by Chater & Morgan[6] as a working model that the synthesis

consisted of "filling-in" of short single-stranded RNA tails on predominantly double-stranded RNA molecules. In the present paper it is shown that, in the case of P. stoloniferum virus S, the virus particle RNA polymerase is a replicase, which, using virus genome double-stranded RNA as template, catalyses the formation of one new double-stranded RNA molecule per virus particle.

METHODS

Preparation and purification of virus and virus RNA. Penicillium stoloniferum, strain ATCC 14586, was grown in shaken flasks for 2 days and virus was obtained from the homogenised mycelium as described previously[7], except that the stage involving co-precipitation with yeast RNA was omitted. Virus preparations were purified by sucrose density gradient centrifugation[8] and separation of P. stoloniferum virus S and P. stoloniferum virus F, was carried out as described by Buck & Kempson-Jones[7]. Virus preparations were finally dialysed against 30 mM tris-HCl buffer, pH 8.0, sterilised by filtration through Millipore GSWP01300 filters and stored at 4°C. Virus RNA was prepared by phenol extraction as described previously[7].

RNA polymerase assays. The RNA polymerase reaction mixture contained 90 mM tris-HCl buffer, pH 8.0; 20 mM magnesium chloride; 1.0 mM EDTA; 0.30 mM ATP; 0.30 mM GTP; 0.30 mM CTP; 0.30 mM ^{3}H-UTP (51 mCi/mmole). Reaction was started by mixing equal volumes of RNA polymerase reaction mixture and virus preparation (E_{260} 0.1 to 1.5) at 30°C. Measurement of incorporation of ^{3}H-UTP into acid insoluble RNA was carried out as described by Chater & Morgan[6].

Isopycnic centrifugation in sucrose density gradients. Virus preparations (1 ml, E_{260} 8) were layered onto pre-formed linear gradients of 25 to 60% $^{w}/_{w}$ sucrose in 30 mM tris-HCl buffer, pH 8.0 (60 ml) and centrifuged at 24,000 rev/min in a Beckman SW 25.1 rotor for 18 h at 4°C. Fractions (0.5 ml) were collected using an ISCO Model D density gradient fractionator and U.V. analyser. Individual fractions containing virus particles were dialysed against 30 mM tris-HCl buffer, pH 8.0 (4 x 5 l).

Isopycnic centrifugation in caesium chloride density gradients.

a) Analytical. Solid caesium chloride was added to virus preparations in order to give a density of 1.36 g/ml. The solutions were then centrifuged to equilibrium (18 to 24 h) at 40,000 rev/min at 22 to 24°C using cells with double-sector filled Epon centrepieces in the AN-F rotor in a Beckman Model E analytical ultracentrifuge. Profiles were obtained with the U.V. Scanner at 265 nm. Average densities of caesium chloride solution were

calculated from refractive indices, measured with a Bellingham and Stanley Abbe High Accuracy '60' refractometer[9]. Virus buoyant densities were calculated by the method of Erikson & Szybalski[10].

b) Preparative. Virus preparations (1ml, E_{260} 8) were layered onto pre-formed linear gradients of 10 to 45% $^{w}/_{w}$ caesium chloride in 30 mM tris-HCl buffer, pH 8.0 (60 ml) and centrifuged for 18 h at 24,000 rev/min at 4°C in a Beckman SW 25.1 rotor. Fractions (0.5 ml) were collected using an ISCO Model D gradient fractionater and U.V. analyser. Densities of caesium chloride solutions were calculated from refractive indices[9].

For isolating P particles from RNA polymerase reactions of virus fractions isolated from isopycnic sucrose density gradients, samples were dialysed against 30 mM tris-HCl buffer, pH 8.0 (5 x 5 l) and then adjusted to a density of 1.36 by addition of solid caesium chloride. The solutions were centrifuged at 45,000 rev/min at 4°C in a Beckman SW 50 rotor for 18 h. Fractions (0.05 ml) were collected from the bottom of gradients using a Buchler density gradient fractionator, and diluted to 0.3 ml prior to measurement of extinction or radioactivity.

For assay of ^{3}H in fractions from caesium chloride density gradients, samples (0.05 ml) were mixed with 2-methoxyethanol (5 ml) and scintillant (10 ml, 0.6% butyl PBD + 5% naphthalene in toluene), prior to scintillation counting.

Measurement of specific activity of RNA in P particles. P particles, from RNA polymerase reactions of virus fractions isolated from sucrose density gradients, were isolated by isopycnic caesium chloride density gradient centrifugation and dialysed against 1.5 mM sodium citrate buffer, pH 7.0, containing 15 mM NaCl (5 x 5 l). Samples (0.5 ml) were made 0.2% with respect to sodium dodecyl sulphate and incubated at 60°C for 20 min in order to disrupt the virus particles and release the virus RNA[11]. Samples were then lyophilised and taken up in water (0.05 ml). After heating to 60°C for 10 min and cooling, samples were analysed by electrophoresis in 4% polyacrylamide gels for 2 h as described by Buck & Kempson-Jones[7], except that the gels were prepared and run in silica tubes. After electrophoresis gels were scanned at 260 nm using a Gilford Model 240 spectrophotometer equipped with a Model 2410-S Linear Transport. The amount of RNA in the gels was quantitated from the areas of the peaks, using gel scans obtained from solutions of P. stoloniferum virus S L particle RNA[7] for calibration.

After removal from the silica tubes the gels were sliced into 1 mm segments using a Mickle gel slicer and the gel slices were digested and

counted for ^{3}H radioactivity as described by Chater & Morgan[6]. In order to monitor the efficiency of counting, samples of the RNA polymerase reaction mixture (without virus) were diluted 1 to 40 with 1 mM tris-HCl buffer, pH 8.0, and 0.05 ml samples were subjected to polyacrylamide gel electrophoresis as above for 1 h. After this time the ^{3}H-UTP had travelled about 4 cm down the gel. The gels were then sliced, digested and counted for ^{3}H radioactivity under the same conditions as for the RNA samples.

RESULTS

Location of RNA polymerase activity in H particles.

P. stoloniferum virus S has been shown previously[7] to consist of a number of particle types, readily separated by isopycnic centrifugation in caesium chloride density gradients, namely, L1 and L2 particles, each containing one molecule of double-stranded RNA of molecular weight 0.94×10^{6} (RNA - S1) and 1.11×10^{6} (RNA - S2) respectively, M1 and M2 particles, each containing one molecule of single-stranded RNA of molecular weight 0.47×10^{6} and 0.56×10^{6} respectively and a range of H particles, containing one molecule of double-stranded RNA (either RNA-S1 or RNA-S2) together with different amounts of single-stranded RNA, which range in sedimentation coefficient from 0 to 15 S. It was postulated that H particles were in the process of replicating their RNA; the different lengths of single-stranded RNA would represent different stages of the replication process. It was therefore important to establish in which of the component particles of this virus the RNA polymerase activity lay. A virus preparation was therefore centrifuged to equilibrium in a preformed linear gradient of sucrose and the gradient was then fractionated. The resultant separation is shown in Fig. 1. Each fraction was dialysed against 30 mM tris-HCl buffer, pH 8.0, and incubated with the RNA polymerase reaction mixture until the reaction was complete (18 h). ^{3}H-UTP incorporation into acid insoluble RNA was measured for each fraction. The results (Fig. 1) show that there was no ^{3}H-UTP incorporation by M particles, but that the RNA polymerase activity lies in the L to H region of the gradient.

In order to ascertain if RNA synthesis takes place in both L and H particles and to establish whether or not RNA synthesis was taking place by filling in of short single-stranded tails on predominantly double-stranded RNA molecules as suggested by Chater & Morgan[6], use was made of the observation that the product of RNA polymerase action remains within

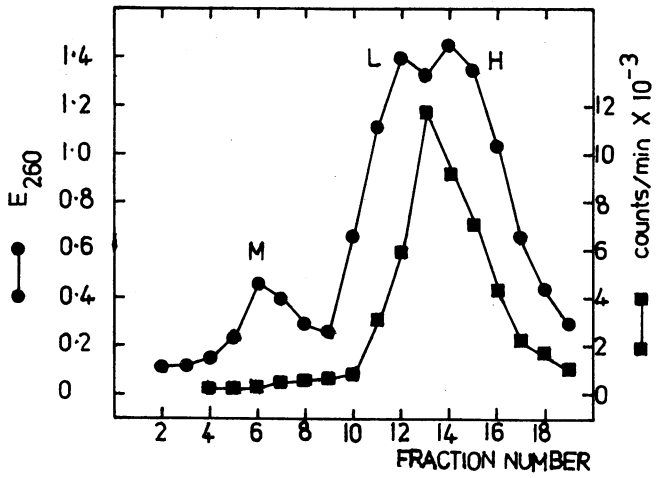

Fig. 1. ^{3}H-UTP incorporation by fractions of P. stoloniferum virus S obtained by isopycnic centrifugation in a sucrose density gradient.

the particles. The buoyant density of the particles in caesium chloride gradient centrifugation should therefore be increased following RNA synthesis. If the reaction consists of merely filling in of single-stranded RNA tails, there would be very little increase in buoyant density. On the other hand if the reaction consisted of synthesising new molecules of double-stranded RNA the increase in buoyant density would be considerable. A virus preparation was therefore incubated with the RNA polymerase reaction mixture and the reaction was allowed to proceed to completion (18 h). The reaction mixture was dialysed against 30 mM tris-HCl buffer, pH 8.0, and solid caesium chloride was added to give an average density of 1.36. One portion of the solution was centrifuged to equilibrium at 45,000 rev/min in the Beckman SW 50 rotor and then fractionated. The extinction at 260 nm and ^{3}H-incorporation of each fraction are shown in Fig. 2. The radioactivity lay entirely in the product particles, designated P. M particles were unchanged in density or amount confirming that these have no polymerase activity. L particles were also unchanged in density or amount, showing that these particles were unreactive and, since there was no ^{3}H-incorporation in this fraction, eliminating the possibility of filling in of single-stranded RNA tails for these particles.

In order to obtain higher resolution a second portion of the

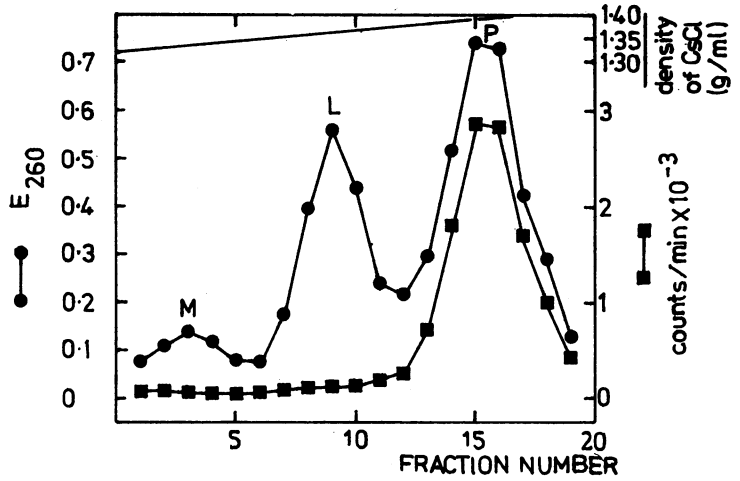

Fig. 2. Fractionation of the products of P. stoloniferum virus S RNA polymerase reaction by isopycnic centrifugation in a caesium chloride density gradient.

solution was centrifuged to equilibrium in the Beckman Model E analytical ultracentrifuge and the u.v. scan, together with a similar profile of unreacted virus, is shown in Fig. 3(a and b). It is clear that RNA synthesis occurred in the whole range of H particles which were converted to the more dense P particles; two classes of P particles, P1 and P2, were resolved with densities of 1.387 and 1.393 respectively. These profiles also confirm that M and L particles were unchanged in density or amount.

Inclusion of S-adenosyl methionine (SAM) in the RNA polymerase reaction mixture at final concentrations of 1 μM, 10 μM or 100 μM had no effect on the rate or final amount of RNA synthesis achieved by P. stoloniferum virus S. Moreover when the products, in reactions containing 1 μM, 10 μM or 100 μM SAM, were analysed by preparative and analytical caesium chloride gradient centrifugation, they were found to be identical to those in which reactions were carried out in the absence of SAM. Profiles identical to those shown in Fig. 2 and Fig. 3(b) were obtained and all the ^{3}H-UTP incorporation was found in P particles. M and L particles were unchanged in density or amount, while the whole range of H particles was converted to P particles.

Nature and location of newly synthesised RNA

When RNA was prepared from P particles, isolated by caesium chloride

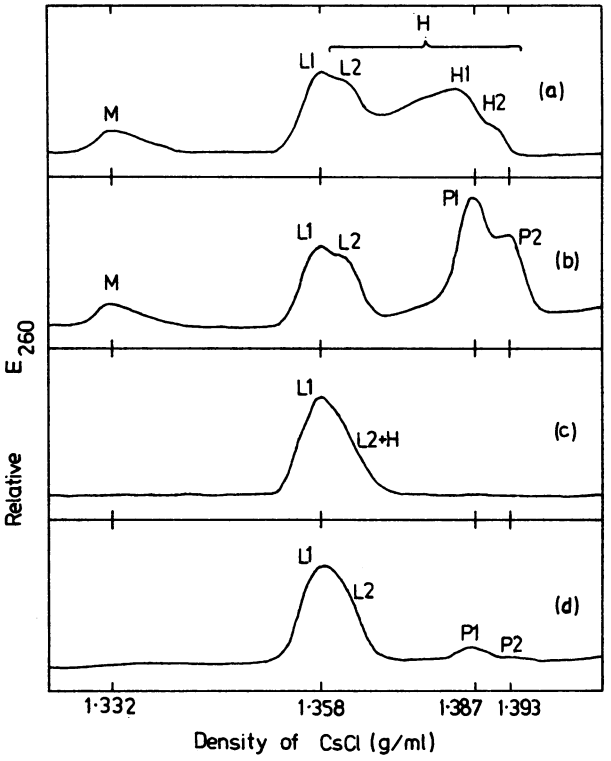

Fig. 3. Analytical equilibrium caesium chloride density gradient centrifugation of P. stoloniferum virus preparations before and after RNA polymerase reaction: U.V. scanner tracings. (a) and (b) unfractionated virus before and after RNA polymerase reaction; (c) and (d) virus fraction 11 from isopycnic sucrose gradient centrifugation (Fig. 1) before and after RNA polymerase reaction.

density gradient centrifugation, and examined by polyacrylamide gel electrophoresis, only two bands of RNA with mobilities of virus genomic double-stranded RNA i.e. S1-RNA and S2-RNA were obtained; the slow moving band of single-stranded RNA found after electrophoresis of RNA from H particles[7] was not detected. After treatment of P particle RNA with pancreatic ribonuclease (200 ng/ml, 2 h, 25°C) in 15 mM sodium citrate buffer, pH 7.0, containing 150 mM NaCl, followed by polyacrylamide gel electrophoresis, the same two RNA bands were unchanged in amount or mobility. When the gels were fractionated and the gel slices were counted for ^{3}H-incorporation, all the radioactivity was found in these two RNA bands. Similar results were obtained with RNA prepared from reactions

containing 1 μM, 10 μM or 100 μM SAM. The experiments confirm the results of Chater & Morgan[6] using RNA prepared from reacted, but unfractionated virus, that the only product of reaction is double-stranded RNA with the same molecular weight as virus genome RNA. When RNA was prepared from separated P1 and P2 particles, and examined by polyacrylamide electrophoresis it was found that P1 particles gave rise only to S1-RNA, while P2 particles gave rise only to S2-RNA.

Amounts of newly synthesised RNA in P particles

In order to quantitate the amount of RNA synthesis achieved by different density classes of H particles, fractions from isopycnic sucrose density gradient centrifugation of the virus (Fig. 1) were dialysed against 30 mM tris-HCl buffer, pH 8.0, and incubated with the RNA polymerase reaction mixture until the reaction was complete (18 h). After dialysis against the tris buffer to remove exess nucleotides, each fraction was analysed by analytical and preparative caesium chloride equilibrium density centrifugation. Fractions were also analysed by caesium chloride analytical ultracentrifugation before polymerase reaction for comparison.

Befor reaction, fraction 10 was found to contain only L1 particles and no detectable H particles. No RNA polymerase activity was detected in this fraction, confirming that the particles containing only double-stranded RNA were inactive in the RNA polymerase assay. Fractions 11 to 19 contained increasing amounts of H particles with progressively increasing densities, and correspondingly decreasing amounts of L particles. After polymerase reaction the products in all cases were P particles; only the H particles had reacted and the L particles were unchanged. The largest increase in density (and consequently the largest amount of RNA synthesis per H particle) was observed in the conversion of the H particles in fraction 11 to P particles. Analytical caesium chloride density gradient centrifugation (Fig. 3c) showed that this fraction, before reaction, consisted mainly of L1 particles (inactive), together with small amounts of L2 particles (inactive) and H particles (active), present as an asymmetry on the more dense side of the L1 peak. The products of RNA polymerase reaction from this fraction were mainly P1 particles (containing RNA-S1) with a small amount of P2 particles (containing RNA-S2); the L particles (about 95% of fraction 11) were unchanged (Fig. 3d). It is clear that since the H particles in this fraction had densities between those of L1 and L2 particles, the single-stranded RNA molecules in these H particles must have molecular weights between 0 and 0.17×10^6 (the difference in molecular weights of RNA-S1 and RNA-S2). As expected, progressively smaller

increases in buoyant densities were observed in the conversion of H particles in fractions from 12 to 19 to P particles.

Because fractions 11 to 19 contained decreasing amounts of L particles which were unchanged after polymerase reaction, in order to measure the specific activity of the RNA product, it was necessary to separate the P particles from the unreacted L particles. P particles were isolated by preparative isopycnic caesium chloride density gradient centrifugation of selected reacted fractions, and RNA isolated from each fraction was examined by polyacrylamide gel electrophoresis. The amount of RNA in each gel was quantitated by scanning at 260 nm and then each gel was fractionated and the gel slices were counted for ^{3}H-incorporation. Approximately equal incorporation on a weight basis was found for RNA-S1 and RNA-S2, but since resolution was incomplete, for greater accuracy they were treated as a single species. The G + C content of P. stoloniferum virus S double-stranded RNA has been estimated to be 53%. Using this value it was calculated that 1 pmole of ^{3}H-UTP incorporated was equivalent to 1.367 ng of RNA synthesised. It was then possible to calculate the % of newly synthesised RNA in the P particles from each fraction. Since each P particle contained a molecule of template double-stranded RNA in addition to the newly synthesised RNA it was possible to calculate the amount of newly synthesised RNA (in daltons) per virus particle. A value of 0.94 x 10^{6} was taken for the molecular weight of template RNA in fraction 11, since this fraction contained mainly RNA-S1 and an average value of 1.03 x 10^{6} was taken for the molecular weight of template RNA in fractions 12 to 19 which contained approximately equal amounts of RNA-S1 and RNA-S2. The results are shown in Table 1.

Table 1

Amounts of newly synthesised RNA in P particles isolated from RNA polymerase reactions of fractions of P. stoloniferum virus S.

Fraction no. (Fig. 1)	Amount of RNA in gel (ng)	pmol of ^{3}H-UTP incorporated	Amount of ^{3}H-RNA (ng)	% of ^{3}H-RNA	Amount of ^{3}H-RNA per virus particle (daltons x 10^{6})
11	233	82.3	113	48.3	0.91
12	647	207	283	43.8	0.90
14	3405	628	858	25.2	0.52
15	4408	471	644	14.6	0.30
16	2578	178	243	9.4	0.19
18	2533	108	148	5.8	0.12
19	1511	61.8	84.5	4.2	0.09

DISCUSSION

The results show that, in the RNA polymerase assay used, only H particles, i.e. those containing both double-stranded RNA and single-stranded RNA, were active; L particles, containing only double-stranded RNA and M particles, containing only single-stranded RNA, were inactive.

The results in Table 1 show that the maximum amount of RNA synthesis occurred in H particles in fraction 11 (Fig. 1), in which the molecular weight of the single-stranded RNA component was the lowest i.e. between 0 and 0.17×10^6 (95% of fraction 11 was comprised of inactive L particles). 48% of the RNA in P particles, isolated from this fraction after polymerase reaction, is newly synthesised. It is clear therefore, that since P particles contain a molecule of template double-stranded RNA, in addition to newly synthesised double-stranded RNA of the same molecular weight, P particles must contain two molecules of double-stranded RNA, one of which is newly synthesised. The difference between the measured amount of newly synthesised RNA per virus particle (0.91×10^6 daltons) and the molecular weight of S1-RNA (0.94×10^6) is probably not experimentally significant. However if the short single-stranded RNA molecules in the H particles of this fraction act as primers for the synthesis of double-stranded RNA, a value slightly lower than 0.94×10^6 daltons would be expected for the amount of newly synthesised RNA. It is clear that, since P1 and P2 particles gave rise only to RNA-S1 and RNA-S2 respectively, P1 particles contain two molecules of RNA-S1 and P2 particles contain two molecules of RNA-S2. Whether the newly synthesised RNA is present in P particles as one molecule of double-stranded RNA or as two complementary single-strands of RNA, which anneal to form double-stranded RNA on extraction, cannot be determined from the present data. The results show that the reaction comes to a halt when one new molecule of double-stranded RNA (or two complementary single-strands of RNA) has been synthesised. This is probably because the newly synthesised RNA remains within the particles, sterically preventing any further RNA synthesis. The synthesis of one new molecule of double-stranded RNA (or its equivalent) by these H particles and the absence of reaction by L particles shows that the reaction does not occur according to the model of Chater & Morgan[6] i.e. by filling in of single-stranded RNA tails on pre-existing double-stranded RNA molecules. Particles containing two molecules of double-stranded RNA have been isolated from *Aspergillus foetidus*[11]; these particles may well have been formed *in vivo* by an RNA polymerase reaction similar to the *in vitro* reaction described here.

Although the detailed mechanism of RNA synthesis is not known, the

results presented here, which show that single-stranded RNA, present in H particles before polymerase reaction, is not present in P particles after reaction, indicate that the reaction may consist of extending and completing single-stranded RNA chains, previously initiated *in vivo*, which are later converted to double-stranded RNA, either within the particles or by annealing of complementary single-strands of RNA on extraction. The progressively lower amount of newly synthesised RNA in P particles derived from H particles in sucrose density gradient fractions 12 to 19 (Table 1) is consistent with their progressively increasing densities and hence additional RNA already synthesised *in vivo*, and supports the view[7] that these particles represent intermediates in double-stranded RNA replication. It is clear that the most dense H particles, labelled H1 and H2 in Fig. 3(a), are those in which the reaction has almost been completed *in vivo*. Only small density changes (Fig. 3a and 3b) and small amounts of RNA synthesis (Table 1, fractions 18 and 19) were observed in their conversion to P1 and P2 particles. It has been pointed out[11] that fungi replicate asynchronously and mycoviruses, which replicate in parallel with their host cells will also multiply asynchronously. Mycovirus preparations may therefore be expected to contain intermediates of replication in addition to mature virions.

The lack of RNA polymerase activity in L and M particles was not due to the absence of the RNA polymerase polypeptide molecules from the capsid of these particles. It has been shown previously[12] that the capsid of *P. stoloniferum* virus S particles consists of 120 molecules of polypeptide S2 (molecular weight 42,500) and one molecule of polypeptide S1 (molecular weight 55,500). The two double-stranded RNA molecules of this virus are sufficient to code only for these two polypeptides[12]. Since S2 is clearly the capsid structural polypeptide it is likely that polypeptide S1 has the RNA polymerase activity. Empty capsids lacking RNA also lack polypeptide S1. No difference was found in the electrophoretic mobilities, molecular weights or proportions of the capsid polypeptides in M,L or H particles[12]. Since the capsids of H particles contains RNA polymerase molecules, the capsids of M and L particles must do also. The absence of RNA polymerase activity in L particles indicates that RNA synthesis cannot be initiated in the *in vitro* assay system; RNA synthesis occurs in this system only in those particles (H particles) in which RNA synthesis has been initiated *in vivo*. It is probable therefore that initiation of RNA synthesis in L particles requires specific initiation factors, not present in the *in vitro* RNA polymerase assay system. The absence of RNA synthesis by M particles

shows that either the RNA polymerase cannot utilise single-stranded RNA as a template and/or that specific initiation factors were required, which were absent from the in vitro assay system.

In the case of cytoplasmic polyhedrosis virus[13,14] and reovirus[15,16] it has been shown that initiation of transcription in vitro is coupled to the 5'-terminal methylation of the nascent RNA, in the presence of the methyl group donor,S-adenosyl methionine (SAM), giving rise to the 5'-terminal structures $m^7G(5')ppp(5')A^mpGp...$ and $m^7G(5')ppp(5')G^mpCp...$ respectively, where m^7G is 7-methylguanosine, A^m is 2'-O-methyladenosine and G^m is 2'-O-methylguanosine. SAM enhanced the rate of transcription by cytoplasmic polyhedrosis virus considerably[13], but stimulation of reovirus transcription by SAM was much less marked[15]. Synthesis of reovirus double-stranded RNA in sub-viral particles, containing reovirus messenger RNA as template[17], would not be expected to be enhanced by SAM since the complementary, negative RNA strands of reovirus have been shown to contain unmethylated 5'-termini[18], and the reaction automatically terminates when the single-stranded RNA template has been converted to double-stranded RNA[17]. In the case of P. stoloniferum virus S inclusion of SAM in the RNA polymerase reaction mixture had no effect on the distribution of RNA polymerase activity among the different particle types, on the rate of RNA synthesis, or on the final amounts of double-stranded RNA and P particles formed. It is clear, therefore, that SAM was unable to initiate RNA synthesis in M or L particles, or to stimulate double-stranded RNA synthesis in H particles.

Double-stranded RNA mycoviruses are unusual in that they replicate in parallel with their hosts and are not released from the host cells (except under special conditions[19]), being carried from one host generation to another. Recently a model for the replication of double-stranded RNA mycoviruses was proposed[20] in which it was suggested that these viruses may replicate by doubling, a method of replication quite different to those of other viruses, including the double-stranded RNA viruses of animals and higher plants[17], but which may be particularly suited to viruses which remain always intracellular. The results of the present paper, which show that the virus particle RNA polymerase catalyses the synthesis of one new molecule of double-stranded RNA per virus particle, i.e. a doubling of the virus RNA, is consistent with this model, although it does not prove it. It is clear that the RNA polymerase of P. stoloniferum virus S is a replicase, which catalyses the synthesis of the two complementary strands of double-stranded RNA in a single virus

particle. This is completely different to the reovirus system, in which the virion RNA polymerase is a transcriptase, catalysing the synthesis of only single-stranded messenger RNA molecules[1,17]; in this case a second sub-viral particle, containing single-stranded RNA as template, is required for the synthesis of virus double-stranded RNA[2,17]. The P. stoloniferum virus S RNA polymerase therefore represents a completely new system for the synthesis of double-stranded RNA. P. stoloniferum virus S and other double-stranded RNA mycoviruses[1] are not members of the virus family reoviridae, defined[21] as "non-enveloped spherical viruses with genomes consisting of several pieces of double-stranded RNA encapsidated within a single virus particle", and which includes viruses of animals and higher plants, such as reovirus, cytoplasmic polyhedrosis virus and wound tumour virus. Although the genome of P. stoloniferum virus S consists of two, and that of other double-stranded RNA mycoviruses several, pieces of double-stranded RNA, each molecule of RNA is separately encapsidated[1,7]. The RNA replicase activity of particles of P. stoloniferum virus S represents a further difference between the reoviridae and a double-stranded RNA mycovirus.

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