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Synthesis of Ribonucleic Acids During the Germination of *Botryodiplodia theobromae* Pycnidiospores

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SUMMARY

During the initial phases of germination of *Botryodiplodia theobromae* pycnidiospores, protein synthesis was initiated in the absence of detectable ribonucleic acid (RNA) synthesis. The lack of RNA synthesis during the early stages of germination was not an artefact due to reduced precursor permeability or to changes in the nucleotide pool. Further studies showed that the synthesis of messenger RNA and transfer RNA began about 30 min after the initiation of germination and that of 5S RNA after 45 min, whereas ribosomal RNA synthesis was not detected until after 1 h of germination. Approximately 45 to 55 % of the messenger-like RNA synthesized during the first hour of germination was devoid of polyadenylate segments.

INTRODUCTION

Fungal spore germination is usually accompanied by rapid increases in the rates of protein and ribonucleic acid (RNA) synthesis. However, the resumption of protein and RNA synthesis may be temporally separate and distinct events during spore germination of some fungi (Van Etten, Dunkle & Knight, 1976). For example, we have reported that in *Botryodiplodia theobromae* pycnidiospores, protein synthesis began immediately after the initiation of germination and continued at a substantial rate in the absence of detectable RNA synthesis (Brambl & Van Etten, 1970). Additional experiments established that dormant *B. theobromae* spores contained endogenous messenger RNA (mRNA) which was responsible for the early protein synthesis. At later stages of germination (about 120 min), RNA synthesis began and increased rapidly during the remainder of the germination process (Brambl & Van Etten, 1970).

One explanation for the lack of detectable RNA synthesis during the initial stages of germination is that such observations were artefactual, resulting from either depressed precursor permeability of the spores or changes in the specific activity of the endogenous precursor pools. In the present report, we show that the inability to detect RNA synthesis early in germination of *B. theobromae* spores was not due to either of these possibilities. However, we did find that if the spores were pulse-labelled with very high concentrations of RNA precursors, a small amount of precursor was incorporated into RNA much earlier in germination than we had previously noted. Further studies revealed that during germination, synthesis of the various classes of RNA was initiated sequentially. That is, the synthesis of mRNA and transfer RNA (tRNA) began about 30 min after the initiation of germination, and that of 5S RNA after 45 min, whereas ribosomal RNA (rRNA) synthesis was not detected until after the first hour of germination.

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METHODS

Materials. [^{3}H]Adenine (20 Ci mmol^{-1}), [^{14}C]adenine (58 mCi mmol^{-1}), [*methyl*-^{3}H]-methionine (11 Ci mmol^{-1}), [^{14}C]leucine (350 mCi mmol^{-1}), and $H_3{}^{32}PO_4$ (carrier free) were from New England Nuclear, Boston, Massachusetts, U.S.A.; poly(d)T-cellulose and polyethyleneimine (PEI)-cellulose F glass plates were from P.L. Biochemicals, Milwaukee, Wisconsin, U.S.A., and Brinkman Instruments, Westbury, New York, U.S.A., respectively. Techniques for growth, harvesting and germination of *B. theobromae* pycnidiospores were modified slightly from those described previously (Brambl & Van Etten, 1970). In these studies, pycnidiospores were germinated in a 14 l fermenter in a defined medium containing 9·8 mM-KNO_3, 2·03 mM-$MgSO_4.7H_2O$, 1·7 mM-NaCl, 3·7 mM-KH_2PO_4, 55·5 mM-glucose. Vogel's trace elements were added to a final concentration of 0·005 × (Vogel, 1956) and a complete vitamin supplement was added at a concentration of 1 g l^{-1}.

Pulse labelling of germinating spores. Samples (200 ml; equivalent to 200 mg ungerminated spores) were removed from the fermenter at various times and quickly harvested by filtration. The spores were resuspended in 50 ml fresh medium containing isotopically labelled precursor(s) (0·5 μCi ^{14}C ml^{-1} or 2 μCi ^{3}H ml^{-1}) and incubated with rapid shaking for 15 min. The spores were harvested by filtration, quickly frozen in liquid nitrogen and stored at −80 °C. In addition, at various times during spore germination, 5 ml samples of spore suspension were incubated with 0·1 μCi ml^{-1} of [^{14}C]leucine, [^{14}C]adenine, or 2 μCi ml^{-1} [*methyl*-^{3}H]methionine to determine their incorporation into trichloroacetic acid (TCA) insoluble material (Brambl & Van Etten, 1970).

Extraction and analysis of RNA. RNA was isolated by a modified form of the procedure described by Brakke & Van Pelt (1970*a*). Spores were combined with 15 g glass beads (1 mm), 5 ml of buffer [0·2 M-ammonium carbonate, 0·002 M-sodium ethylenediaminetetraacetate (EDTA), 0·2 % sodium dodecyl sulphate (SDS), pH 9·0] containing bentonite (100 to 200 μg ml^{-1}), and 5 ml 80 % (w/v in water) phenol containing 0·01 % 8-hydroxyquinoline. The cells were disrupted in a Braun model MSK mechanical homogenizer (Bronwill Scientific Co., San Mateo, California, U.S.A.) by shaking for two 60 s periods at 4000 rev/min while being cooled with solid CO_2. The phenol and aqueous phases were separated by centrifuging, the aqueous phase was removed, and the phenol phase was extracted again with 5 ml fresh aqueous buffer at 22 °C. After centrifuging, the two aqueous phases were combined and extracted with 10 ml fresh phenol solution. Nucleic acids were precipitated from the aqueous phase by adding 2 vols cold ethanol in the presence of bentonite (100 μg ml^{-1}). The nucleic acids were dissolved in a small volume of 1 × SSC buffer (SSC buffer is 0·15 M-sodium chloride/0·015 M-sodium citrate, pH 7·0) containing bentonite, and dialysed overnight against 1000 vols of the same buffer, or desalted on a 1·8 × 25 cm column of Sephadex G-25 equilibrated with 1 × SSC. The RNA was reprecipitated with ethanol and dissolved in an appropriate buffer just before analysis. When RNA was isolated from spores that had been pulse-labelled with [*methyl*-^{3}H]methionine, tRNA was deacylated and the radioactive material tested for ribonuclease sensitivity.

The RNA was analysed on linear-log sucrose density gradient columns (Brakke & Van Pelt, 1970*b*) equilibrated with 1 × SSC buffer and by polyacrylamide gel electrophoresis. Gradient columns were centrifuged using a Beckman (Beckman Instruments, Fullerton, California, U.S.A.) SW41 rotor for 10 h at 37000 rev./min at 14 °C; and scanned photometrically with an ISCO density gradient fractionator (Instrumentation Specialities, Lincoln, Nebraska, U.S.A.). For determining radioactivity, 0·25 ml fractions were collected, diluted with 1·5 ml water and counted as described previously (Van Etten *et al.*, 1973).

Gel electrophoretic analysis was performed on 3 and 10% polyacrylamide gels (5% *N'*,*N'*-methylenebisacrylamide cross-linked; 0·6 × 9·0 cm) containing 0·5% agarose (3% gels), in a buffer containing 0·04 M-Tris, 0·033 M-sodium acetate, 0·001 M-EDTA, 0·2% SDS, pH 7·2. After pre-electrophoresis for 3 h at 6 mA/gel, 0·05 to 0·10 ml samples in 10% sucrose were subjected to electrophoresis at room temperature for 4 h at 5 mA/gel. The gels were scanned at 260 nm with a Gilford Model 2410 linear transport system (Gilford Instrument Laboratories, Oberlin, Ohio, U.S.A.), coupled to a Beckman DU spectrophotometer. Radioactivity was determined by eluting the radioactive material from 1 mm gel slices (Zaitlin & Hariharasubramanian, 1970) and counting as described previously (Van Etten *et al.*, 1973). Polyacrylamide gels and sucrose density gradients were routinely run with 0·75 and 1·0 E_{260} units of RNA, respectively.

Extraction and fractionation of nucleotide pools. Samples of pulse-labelled spores (25 mg) were mixed with 5 ml freshly prepared 10% TCA for 60 min at 0 °C. After centrifuging, the supernatant was extracted with 10 vols chilled ether and the aqueous phase was lyophilized. Samples containing nucleoside triphosphate standards were chromatographed on PEI-cellulose thin-layer plates using the technique described by Cashel, Lazzarini & Kalbacher (1969). On completion of the separation, the ribonucleoside triphosphate standards were located with ultraviolet light, scraped from the plate, and the co-migrating radioactivity determined. The concentration of adenosine triphosphate (ATP) in germinating spores was determined by the luciferin–luciferase assay (Addanki, Sotos & Rearick, 1966).

Other procedures. Polyadenylate-containing RNA [polyA(+)RNA] was separated from total RNA by chromatography on a poly(d)T-cellulose column using a procedure modified from that of Nakazato & Edmonds (1974). After eluting the polyA(−)RNA with a buffer containing 0·3 M-NaCl, 0·02 M-EDTA, 0·2% SDS, and 0·01 M-Tris, pH 7·5, at 25 °C, the polyA(+)RNA was eluted with the same buffer minus 0·3 M-NaCl at 65 °C.

To determine if [^{3}H]adenine incorporation into tRNA was due solely to 3′-hydroxyl addition, the 2 M-LiCl-soluble RNA from pulse-labelled spores was subjected to venom phosphodiesterase digestion as described previously (Roheim, Knight & Van Etten, 1974). RNA concentrations were determined by assuming 1 mg RNA ml^{-1} had an E_{260} of 24.

RESULTS

Kinetics of RNA and protein synthesis

Botryodiplodia theobromae pycnidiospores germinated synchronously and at a reproducible rate. Germ tubes were first observed after 2·5 h of germination and after 5 h 96% of the spores had germ tubes (Fig. 1*a*). The kinetics of [^{14}C]adenine, [^{14}C]leucine, or [*methyl*-^{3}H]methionine incorporation into the germinating spores are shown in Fig. 1(*b*). Substitution of [^{14}C]uracil or $H_3{}^{32}PO_4$ for [^{14}C]adenine gave the same pattern. The results indicate that protein synthesis began immediately on initiation of germination and continued at a substantial rate in the absence of significant RNA synthesis; the incorporation of less than 4 pmol adenine into 5 mg spores was detected in the first 15 min compared with 469 pmol between 225 and 240 min. [*methyl*-^{3}H]Methionine, a precursor of both protein synthesis and post-transcriptional methylation of RNA, was incorporated into spores in a manner similar to [^{14}C]leucine (Fig. 1*b*). Previous studies by Brambl & Van Etten (1970) and Van Etten, Roker & Davies (1972) showed that the incorporation of [^{14}C]leucine during the early stages of germination reflected genuine protein synthesis.

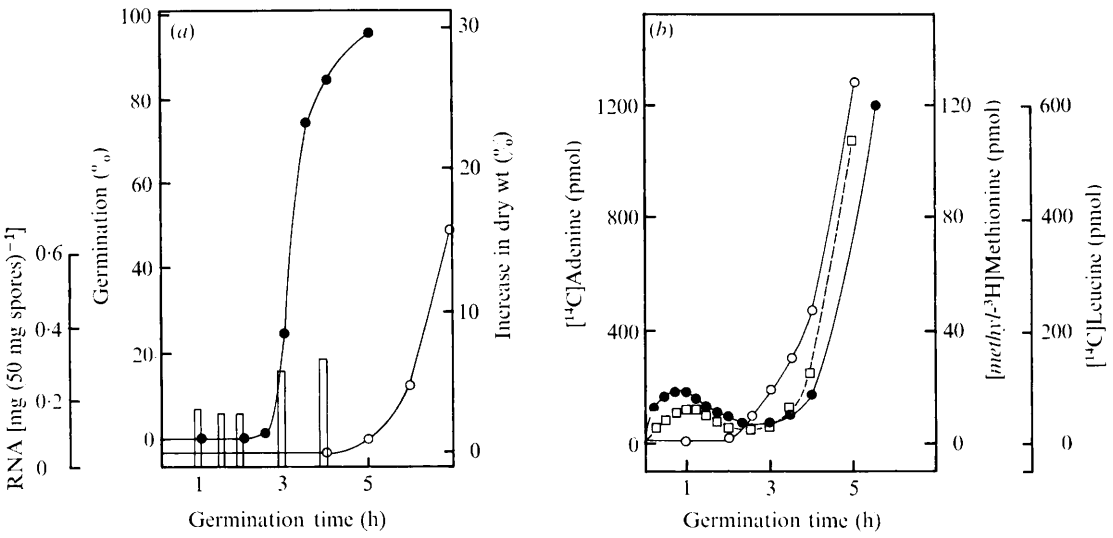

Fig. 1. Characteristics of *B. theobromae* spore germination. (*a*) Germination (●), increase in dry weight (○), and total RNA content (bars) plotted as a function of germination time. (*b*) Incorporation of [^{14}C]adenine (○), [^{14}C]leucine (●), and [*methyl*-^{3}H]methionine (□) into 5 mg germinating spores. The points plotted represent the amount of isotope incorporated during each 15 min pulse period and the connecting lines reflect changes in the capacity for incorporation.

Table 1. *Distribution of ^{32}P incorporated into ribonucleoside triphosphates during B. theobromae spore germination*

Time of pulse (min)	$H_3{}^{32}PO_4$ incorporated into ribonucleoside triphosphate [d.p.m. (mg spores)$^{-1}$]				
	ATP	UTP	GTP	CTP	Total
0–15	45904	15472	7024	20464	88864
45–60	34752	12656	5096	16784	69288
105–120	45360	34136	8392	17648	105536
165–180	117992	44944	22736	32736	218408
225–240	319008	128224	63920	163032	764184
Ratio ^{32}P incorporated at 225–240 min/0–15 min	6·95	8·29	9·1	7·97	8·59

Ribonucleotide pools

It is possible that the low level of RNA synthesis observed during the first 2 h of germination was an artefact resulting from depressed permeability of the spores to precursors or that endogenous pools within the spores were at such concentrations that they diluted out the labelled precursors as they were taken up. To investigate the first possibility, spores were pulse-labelled with $H_3{}^{32}PO_4$ or [^{3}H]adenine for 15 min periods throughout germination, the nucleotides were extracted, and ribonucleoside triphosphates were fractionated by thin-layer chromatography. Spores pulse-labelled from 0 to 15 min, 45 to 60 min and 105 to 120 min readily incorporated $H_3{}^{32}PO_4$ to approximately the same extent into all four ribonucleoside triphosphates (Table 1). After 2 h, incorporation increased rapidly so that there was a ninefold increase in incorporation for spores pulsed between 225 and 240 min compared with those pulsed between 0 and 15 min. The incorporation of [^{3}H]adenine into ATP at various stages of germination followed a similar pattern (Table 2). During this same per-

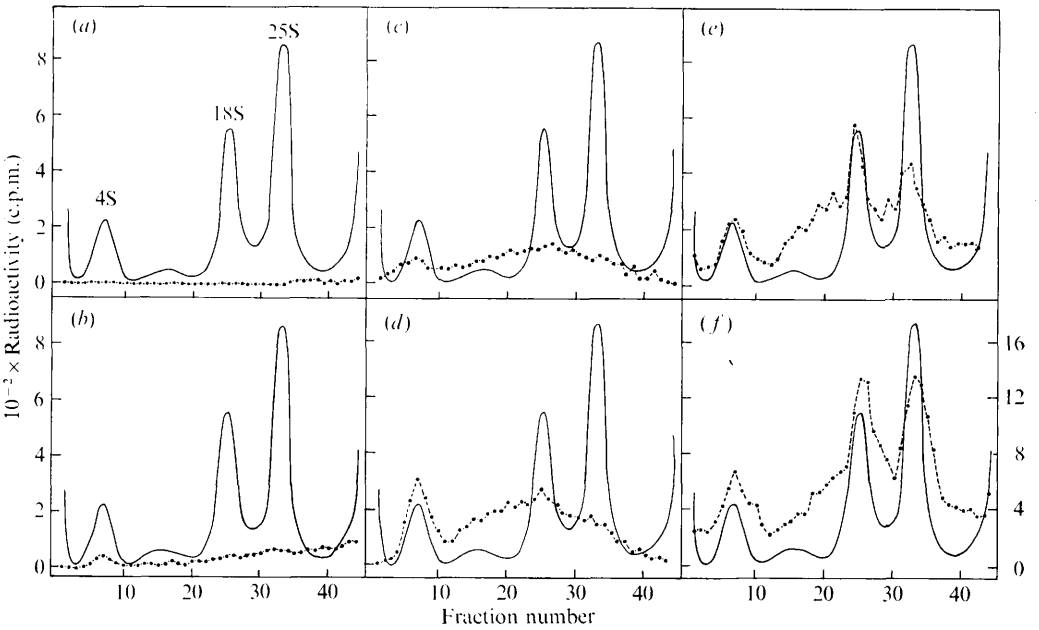

Fig. 2. Sucrose density gradient analysis of RNA isolated from *B. theobromae* spores pulse-labelled with [^{3}H]adenine (●) for 15 min periods at various times during germination: (*a*) 0–15 min, (*b*) 15–30 min, (*c*) 30–45 min, (*d*) 45–60 min, (*e*) 75–90 min, (*f*) 105–120 min. The continuous line shows the variation in extinction at 254 nm: the peaks corresponding to tRNA (4S) and 18S and 25S RNAs are indicated in (*a*). Note that the different radioactivity scale only relates to (*f*).

Table 2. *The ATP concentration and [^{3}H]adenine and ^{32}P incorporation into the endogenous ATP pool during B. theobromae spore germination*

Time of pulse (min)	ATP [nmol (mg spores)$^{-1}$]	[^{3}H]ATP [d.p.m. (mg spores)$^{-1}$]	[^{3}H]ATP [d.p.m. (nmol ATP)$^{-1}$]	[^{32}P]ATP* [d.p.m. (nmol ATP)$^{-1}$]
0–15	2·75	80600	29309	16692
45–60	[2·50	58800	23520	13900
105–120	2·75	79100	28764	16494
165–180	3·35	243999	72835	35221

* Calculated from the data in Table 1.

iod, [^{14}C]adenine incorporation into RNA increased about 123-fold. Thus, while the amount of label in the ribonucleoside triphosphate pool increased after 2 h, this increase was probably due to the increased demand produced by the rapid increase in RNA synthesis. If this assumption is correct, the relative concentrations of total ribonucleoside triphosphates might also increase during germination. Experiments in which the total concentration of ATP in the spores was monitored by the luciferin–luciferase assay indicated that the total concentration of ATP remained relatively stable during the first 2 h of germination (Table 2). However, as the isotope incorporation into the ribonucleoside triphosphate pool began to increase (3 h), the total ATP concentration also increased (Table 2).

Synthesis of mRNA and rRNA during spore germination

To determine the classes of RNA synthesized during the earliest stages of germination, total RNA was isolated from spores pulse-labelled with either [^{3}H]adenine or [*methyl*-^{3}H]-

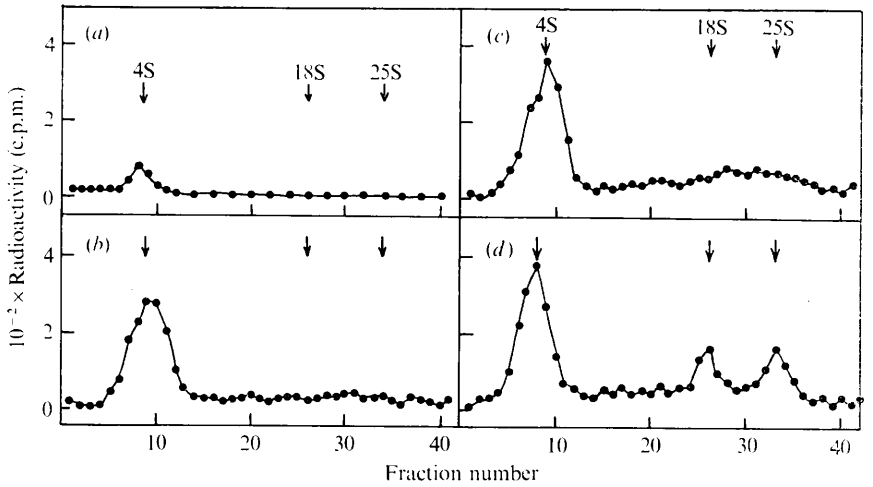

Fig. 3. Sucrose density gradient analysis of RNA isolated from *B. theobromae* spores pulse-labelled with [*methyl*-^{3}H]methionine for 15 min periods at various times during germination: (*a*) 0–15 min, (*b*) 30–45 min, (*c*) 45–60 min, (*d*) 75–90 min. The position at which tRNA and 18S and 25S RNAs sedimented is indicated by the arrows.

Table 3. *Distribution of newly-synthesized RNA from B. theobromae spores into polyA(−)-RNA and polyA(+)RNA after chromatography on a poly(d)T-cellulose column*

The RNA was isolated from spores pulse-labelled with [^{3}H]adenine for 15 min periods during the germination process.

Time of pulse (min)	Radioactivity (c.p.m.)		Percentage of total radioactivity incorporated into RNA	
	PolyA(+)RNA	PolyA(−)RNA	PolyA(+)RNA	PolyA(−)RNA
30–45	2280	5472	29·4	70·6
45–60	3192	8664	26·9	73·1
75–90	4560	26448	14·7	85·3
105–120	5472	41040	11·8	88·2
165–180	32376	384864	7·8	92·2
225–240	39672	538536	6·9	93·1

methionine for 15 min periods and analysed by sucrose density gradient centrifugation. No incorporation of adenine into RNA was detected in spores pulsed from 0 to 15 min (Fig. 2*a*). A small amount of radioactivity was present in the tRNA region (3 to 7S) and a heterogeneous distribution of radioactivity was detected in the 18 to 30S region of the gradient in spores pulsed from 15 to 30 min (Fig. 2*b*). Only radioactive material which sedimented like mRNA (7 to 25S) and tRNA (3 to 7S) was present in spores pulsed from 30 to 45 min (Fig. 2*c*) and 45 to 60 min (Fig. 2*d*). Spores pulsed from 75 to 90 min (Fig. 2*e*) also contained radioactive peaks characteristic of rRNA (18S and 25S). During later pulse periods, the majority of the radioactivity was incorporated into rRNA. Labelling patterns similar to those obtained during the pulse periods 30 to 45 min and 45 to 60 min were also observed when spores were continuously incubated with [^{3}H]adenine during the first hour of germination.

Radioactive sedimentation patterns of RNA isolated from spores pulse-labelled with

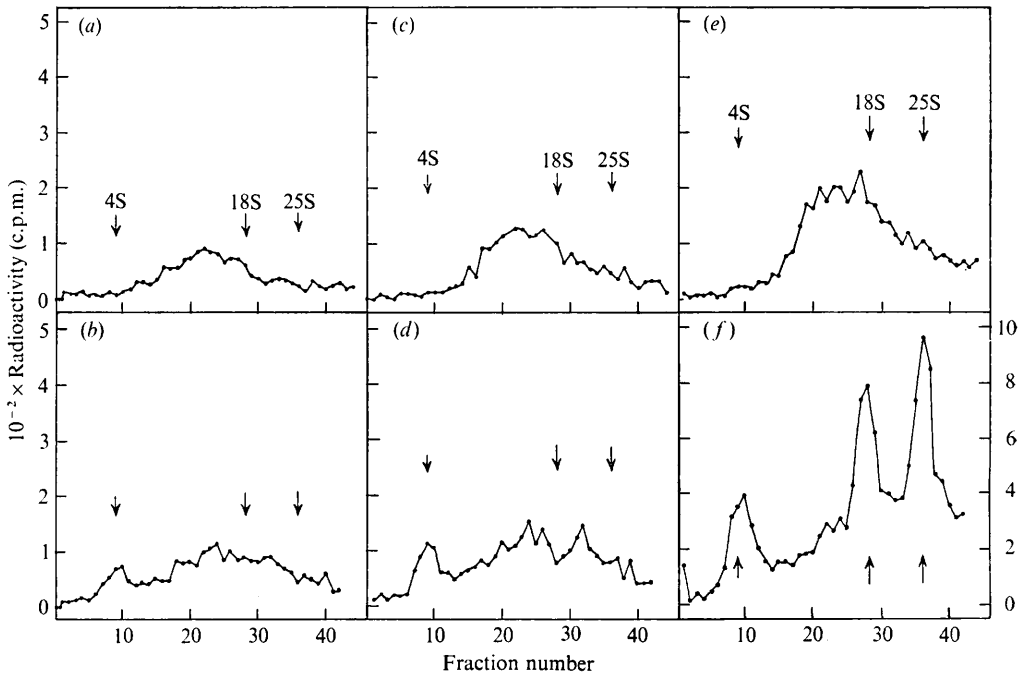

Fig. 4. Sucrose density gradient analysis of polyA(+)RNA (*a*, *c*, *e*) and polyA(−)RNA (*b*, *d*, *f*) isolated from *B. theobromae* spores pulse-labelled with [^{14}C]adenine for 15 min periods at various times during germination: (*a*, *b*) 30–45 min, (*c*, *d*) 45–60 min, (*e*, *f*) 105–120 min. The position at which tRNA and 18S and 25S RNAs sedimented is indicated by the arrows. Note that the different radioactivity scale only relates to (*f*).

[*methyl*-^{3}H]methionine are shown in Fig. 3. Radioactive material sedimenting in the region of tRNA was observed for all pulse periods. In contrast, radioactive peaks sedimenting in the region of the rRNAs were not observed in spores pulse-labelled earlier than 75 to 90 min (Fig. 3*d*).

The distribution of [^{3}H]adenine incorporated into polyA(+)RNA and polyA(−)RNA at various times during germination is shown in Table 3. About 29 % of the radioactivity was incorporated into poly(+)RNA between 30 and 45 min after the start of germination; the percentage of radioactivity in the polyA(+)RNA fraction steadily decreased with germination time until it was only 7 % of the total radioactivity in pulse-labelled RNA between 225 and 240 min. The decrease in the percentage of adenine incorporated into polyA(+)RNA with germination time was the result of the rapid increase in rRNA synthesis after the first hour of germination rather than the lack of synthesis of poly(+)RNA. As reported in Table 3, the rate of polyA(+)RNA synthesis increased approximately 12-fold between 1 and 4 h; in contrast, radioactivity which electrophoresed in the region of large rRNA increased about 100-fold during the same period.

Sucrose density gradient centrifugations of polyA(+)RNA and polyA(−)RNA synthesized between 30 and 45 min and 45 and 60 min are shown in Fig. 4 (*a* to *d*). Except for a small amount of radioactivity which sedimented in the region of tRNA in the polyA(−)-RNA fraction, all of the radioactivity in both fractions sedimented in a heterogeneous fashion (about 7S to 25S). In contrast, the majority of the radioactivity in the polyA(−)-RNA fraction isolated from spores pulse-labelled between 105 and 120 min consisted of

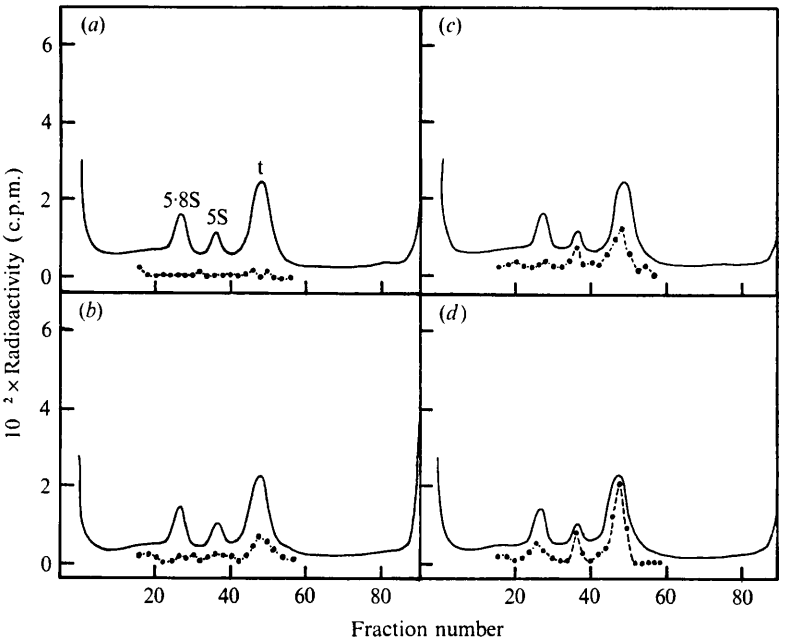

Fig. 5. Electrophoretic analysis of small molecular weight RNAs (5·8S, 5S and tRNA) on 10% polyacrylamide gels. The RNA was isolated from *B. theobromae* spores pulse-labelled with [^{3}H]-adenine (●) for 15 min periods at various times during germination: (*a*) 0–15 min, (*b*) 30–45 min, (*c*) 45–60 min, (*d*) 75–90 min. The continuous line shows the variation of extinction at 260 nm. Direction of migration is from left to right.

rRNA (Fig. 4*f*). Thus, these data suggest that in addition to the synthesis of polyA(+)-mRNA, much of the polyA(−)RNA synthesized during the first hour of germination is probably mRNA devoid of polyadenylate segments.

Synthesis of 5·8S, 5S and tRNA

As noted in Figs 2 and 3, [^{3}H]adenine and [*methyl*-^{3}H]methionine were incorporated very early in germination into an RNA fraction which sedimented at 3 to 7S. Accordingly, RNA was subjected to electrophoresis on 10% polyacrylamide gels to resolve the three small molecular weight RNA species (5·8S, 5S and tRNA) present in this region (Knight & Van Etten, 1976). Incorporation of [^{3}H]adenine into tRNA was first detected in spores labelled between 30 and 45 min after the start of germination (Fig. 5*b*); this incorporation increased with germination time. Separate experiments established that the [^{3}H]adenine incorporated into the tRNA in the first hour of germination was due to *de novo* synthesis of the tRNA and not the result of adenine addition to the 3′-hydroxy groups. Adenine incorporation into 5S RNA and 5·8S RNA was first detected after 45 to 60 min and 75 to 90 min, respectively (Figs 5*c*, *d*). Since 5·8S RNA is probably synthesized from the same precursor RNA as rRNA (Udem & Warner, 1972), this experiment provides additional evidence that rRNA synthesis began after 1 h of germination.

When RNA was isolated from spores pulse-labelled with [*methyl*-^{3}H]methionine for 15 min periods, radioactivity was detected in tRNA during the first 15 min of germination and at all subsequent times (Fig. 6). Since methylation of tRNA occurred before there was

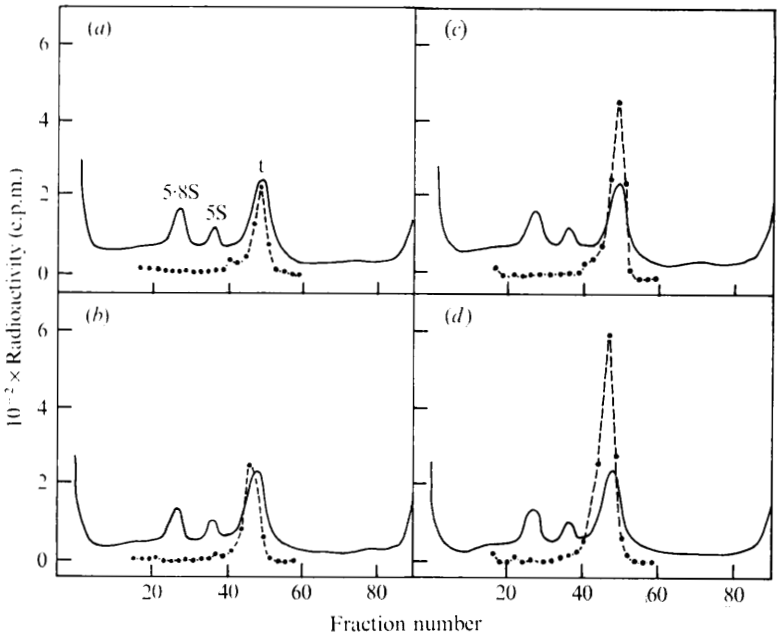

Fig. 6. Electrophoretic analysis of small molecular weight RNAs (5·8S, 5S and tRNA) on 10% polyacrylamide gels. The RNA was isolated from *B. theobromae* spores pulse-labelled with [*methyl*-^{3}H]methionine (●) for 15 min periods at various times during germination: (*a*) 0–15 min, (*b*) 15–30 min, (*c*) 30–45 min, (*d*) 45–60 min. The continuous line shows the variation of extinction at 260 nm. Direction of migration is from left to right.

any detectable tRNA synthesis, the results suggest that methylation of pre-existing tRNA occurre d during the first few minutes of germination.

DISCUSSION

The results support our previous observation that protein synthesis begins immediately after the initiation of germination and before RNA synthesis (Brambl & Van Etten, 1970). Furthermore, discernible RNA synthesis was very low during the first 2 h of germination and then increased rapidly during the later stages of germination. This low level of RNA synthesis during the first 2 h was not due to the inability of the spores to take up the precursor and convert it to ribonucleoside triphosphates, or to changes in the size of the endogenous pools. However, the data do not preclude the possibility that the spores contain partitioned ribonucleotide pools.

We found that some RNA synthesis could be detected much earlier (about 30 min) in the germination process than we had previously reported (about 120 min, Brambl & Van Etten, 1970). The reason for this difference is that in the present study we used much higher concentrations of radioactive precursors and analysed isolated RNA. Analysis of the various classes of RNA isolated from pulse-labelled germinating spores of *B. theobromae* revealed that their initial synthesis occurred in a sequential manner. A small amount of mRNA and tRNA synthesis was detected as early as 30 min and 5S RNA synthesis at 45 min; rRNA synthesis was not detected until after 1 h of germination. A sequential initiation of RNA synthesis has been reported during the germination of other fungal spores. Hollomon (1970) reported that the synthesis of tRNA began first, followed by the synthesis of

mRNA and, lastly, rRNA during conidial germination of *Peronospora tabacina*. During germination of *Aspergillus oryzae* conidia the synthesis of rRNA began immediately, followed by tRNA and, lastly, mRNA (Ono, Kimura & Yanagita, 1966; Tanaka, Ono & Yanagita, 1966). In both these fungi as well as in *B. theobromae* it was at least 60 min after the start of the germination process before the synthesis of all classes of RNA was detected. In contrast, similar studies on the germination of *Rhizopus stolonifer* sporangiospores indicated that the synthesis of all classes of RNA occurred within a few minutes after the spores were placed on a germination medium (Roheim *et al.*, 1974).

Our results also indicate that methylation of tRNAs occurred immediately after *B. theobromae* spores were placed in a germination medium. Since methylation occurred before *de novo* synthesis of tRNA presumably methylation of pre-existing tRNAs in the dormant spore had occurred. Previous studies have demonstrated quantitative and qualitative changes in tRNA isoaccepting species during germination of other fungal spores (Horikoshi, Ohtaka & Ikeda, 1969; Merlo, Roker & Van Etten, 1972). In addition, at least some tRNAs from *Neurospora crassa* conidia are undermethylated (Wong, Scarborough & Borek, 1971). However, the significance of these observations to fungal spore germination is unknown.

Because mRNA and tRNA were selectively synthesized during the first hour of spore germination, it was possible to estimate the percentage of mRNA devoid of polyadenylate segments. Between 30 and 60 min of germination approximately 30 % of the [^{3}H]adenine incorporated into RNA was isolated with the polyA(+)RNA fraction (Table 3), and 15 to 25 % of the label was in the tRNA fraction. Therefore, in the absence of significant rRNA synthesis, the majority of the remaining RNA synthesized (45 to 55 %) is probably mRNA devoid of polyadenylate sequences, i.e. polyA(−)mRNA. This value is similar to the values reported for polyA(−)mRNA in mammalian cells (Milcarek, Price & Penman, 1974; J. Greenberg, personal communication) and in sea urchin embryos during early development (Nemer, Graham & Dubroff, 1974). The polyA(−)mRNA fraction from *B. theobromae* exhibited characteristics similar to those of polyA(+)RNA; it had a paucity of methylated ribonucleotides, was ribonuclease susceptible, and sedimented in a heterogeneous fashion on sucrose density gradients.

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