Ribonucleic Acid Synthesis During Fungal Spore Germination

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SUMMARY

Spores of several fungi were examined in an effort to understand the role of RNA in the biochemical events occurring early in germination. RNA synthesis appeared to be an essential requirement for germination in *Neurospora crassa* and *Aspergillus nidulans* but not in *Alternaria solani* and *Peronospora tabacina*. Gross changes in the relative amounts of the various molecular species of RNA were not detected during germination of *P. tabacina* and *N. crassa* spores. Pulse-labelling of the germinating spores revealed that, in *N. crassa*, ribosomal and soluble RNAs were the major species being synthesized, whereas in *P. tabacina* label was incorporated into soluble RNA and an unstable heterodisperse RNA, but apparently not into ribosomal RNA. This pattern of RNA synthesis in germinating *P. tabacina* spores is similar to that found in cleaving embryos of certain animals where development is also rapid and, like germinating *P. tabacina* spores, can occur when RNA synthesis is inhibited.

INTRODUCTION

Protein synthesis appears essential for the germination of fungal spores (Gottlieb, 1966). However, studies with *Peronospora tabacina* conidia revealed that RNA synthesis, although occurring before germ-tube emergence, was not required for germination (Hollomon, 1969). Although several workers have examined RNA synthesis in germinating fungal spores (for review see Gottlieb, 1966), the importance of this synthesis in the germination of species other than *P. tabacina* is largely unknown. The importance of this synthesis in the germination of spores of five fungi has been assessed. Results show that species differ not only in their need to synthesize RNA, but also in the types of RNA being synthesized.

METHODS

Preparation and germination of spores

Peronospora tabacina Adam. Conidia were collected and prepared as described previously (Hollomon, 1969) except that chloramphenicol (100 µg./ml.) was added to the germination medium to limit bacterial contamination. (The rate of germination was unaltered by this concentration of chloramphenicol.) Conidial suspensions (2.0×10^5 /ml.) were incubated at 15° and germ tubes first appeared after 60 min.

Neurospora crassa Shear and Dodge. Using cold sterile distilled water, conidia were washed from 4 day cultures grown on agar slopes of the medium described by Ryan (1950). Mycelial fragments were removed by filtration through glass wool, and when conidial suspensions (1×10^6 /ml.) were incubated at 30° in Ryan's medium, germ tubes first appeared after 2 h.; after 8 h. 80% of the conidia had germinated.

Aspergillus nidulans (Eidam) Wint. Using 0.02 % (v/v) Teepol, conidia were washed from 7 day cultures grown on agar slopes of Czapek-Dox medium (pH 6.8) modified by the addition of biotin (10 µg./l.) and trace element solution (Sussman, 1966) (1.0 ml./l.). Suspensions were filtered to remove mycelial fragments and washed once with cold sterile distilled water. When suspensions (4×10^6 conidia/ml.) were incubated at 37° in the modified Czapek-Dox medium, germ tubes appeared after 4 h.; at 8 h. 80 % of the conidia had germinated.

Alternaria solani (Ellis and Martin) Sorauer. Sporulation of this fungus is stimulated by alternating periods of light and dark (Lukens, 1963). Cultures were, therefore, grown at 24° on potato glucose agar and placed on a windowsill where they received natural daylight. Conidia were washed from 14 day cultures with cold sterile distilled water and mycelial fragments removed by filtration through glass wool. Conidial suspensions (2×10^5 /ml.) were incubated at 26° in the modified Czapek-Dox medium used for *Aspergillus nidulans*; germ tubes first appeared after 60 min.; at 6 h. 90 % of the conidia had germinated.

Puccinia graminis Pers. f. sp. *tritici* Eriks and E. Henn. Uredospores (strain ANZ 34-1,2,3,6,7 kindly supplied by Dr N. H. Luig, University of Sydney, N.S.W., Australia) were stored under vacuum in a refrigerator. To initiate germination 5 mg. of uredospores were heat-shocked for 5 min. at 40° (Bromfield, 1964) and then suspended in 2.5 ml. 0.01 % (v/v) Tween 20. When shaken at 26° in 50 ml. conical flasks germ tubes appeared within 30 min.; maximum germination, which never exceeded 60 %, occurred after 2 h.

Germination was considered complete when the first sign of a germ tube was noted and, unless stated otherwise, all species were germinated without shaking in 5.0 cm. diameter Petri dishes containing a final volume of 2.5 ml. Except for the *Peronospora tabacina* medium, which was not sterilized, all media were autoclaved at 117° for 15 min.

RNA extraction from germinating Peronospora tabacina and Neurospora crassa conidia

Germination was stopped by cooling with ice and conidia were collected on membrane filters (Oxoid). Bentonite (7.0 mg.) was added and the conidia were then transferred to a chilled mortar containing solid CO₂, sand, and sufficient 20 % (w/v) sodium dodecyl sulphate (SDS) to give a final concentration of 1 % (w/v) SDS. Conidia were broken by grinding (microscopic examination showed breakage always exceeded 80 %) and extracted with 3.0 ml. 0.05 M-tris + HCl (pH 8.0) containing 5.0 mM-MgCl₂. The broken spore suspension was extracted three times with tris-saturated phenol containing 0.05 M-EDTA and 0.5 % (w/v) SDS, the first phenol extraction being carried out by shaking for 3 min. at 60°. Subsequent phenol extractions and further purification were carried out as described previously (Hollomon, 1969).

Fractionation of RNA by polyacrylamide gel electrophoresis

Nine cm. polyacrylamide gels (2.4 % w/v) were prepared in Perspex tubes (1/4 in. internal diameter $\times$ 5 in. long) by the method of Loening (1967) except that 10 % (v/v) glycerol and 1 % (w/v) agarose were added to facilitate slicing the gels. RNA samples were layered directly on to the gels in tris + acetate buffer (final concentration 0.045 M-tris + HCl; 0.02 M-Na acetate; 2 mM-sodium EDTA; acetic acid was used to adjust to pH 7.3. at 24°) containing 15 % (v/v) glycerol, and electrophoresis was carried

out for 150 min. at 5 mA/gel and 10 V/cm. using tris + acetate buffer containing 10 % (v/v) glycerol.

After electrophoresis gels were stained in 0.025 % (w/v) Azure B in 0.065 M-citrate + phosphate buffer (pH 4.6), de-stained overnight in distilled water, and scanned in a Joyce Loeb Chromoscan with a red (620 nm.) filter. Under these conditions direct proportionality between absorbance and amount of nucleic acid did not exist, and therefore quantitative estimation of the RNA was not possible. After scanning, gels were frozen with solid CO₂ and sliced into 1 mm. sections with a McIlwain gel slicer (Mickle Engineering, Gomshall, Surrey). Two sections were placed into each scintillation vial and hydrolysed with 0.5 ml. hyamine for 30 min. at 50°. To accommodate the water present in the samples six drops of Triton X 100 were added to each vial from a Pasteur pipette and the radioactivity was counted in a Packard Tri-carb Liquid Scintillation Spectrometer using 2,5 diphenoxazole (6.0 g./l. toluene) as scintillant.

Preparation of ribosomes from Peronospora tabacina conidia

Germinated conidia were augmented with dormant conidia to provide sufficient material for manipulation. Conidia were collected on a membrane filter and transferred to a chilled mortar containing solid CO₂, sand and 28 mg. sodium deoxycholate. Conidia were broken by grinding and upon thawing were extracted with 5.0 ml. buffer (0.01 M-tris + HCl (pH 7.8); 0.01 M-Mg acetate; 0.06 M-KCl; 0.006 M-mercaptoethanol (KMT buffer)). Sand was sedimented from this extract by centrifugation for 1 min. at 100 g and the supernatant fluid incubated at 37° for 20 min. to permit digestion of messenger RNA. Mitochondria and larger particles were sedimented by centrifugation for 10 min. at 10,000 g and the supernatant fluid further centrifuged for 2 h. at 117,000 g. The ribosomal pellet was suspended in 2.0 ml. KMT buffer and centrifuged for 90 min. at 117,000 g to yield washed ribosomes. These ribosomes gave a characteristic u.v. spectrum with a $E_{1\text{ cm.}}^{260}:E_{1\text{ cm.}}^{230}$ of 1.4, indicating the presence of about 20 % contaminating protein (Peterman, 1964). Dialysis for 90 min. against 0.05 M-tris + HCl (pH 7.8) containing 0.01 M-sodium EDTA resulted in dissociation of the ribosomes into two subunits which could be fractionated on a 5 to 20 % (w/v) sucrose density gradient by centrifugation for 2 h. at 37,000 rev./min. (Spinco S.W. 39 rotor).

RNA present in these ribosome preparations was fractionated by polyacrylamide gel electrophoresis using the procedure described above, except that in the gels and the electrophoresis buffer glycerol was replaced by 0.2 % (w/v) SDS. Ribosomes were disrupted in KMT buffer (without mercaptoethanol) containing 0.5 % (w/v) SDS and 10 % (w/v) sucrose and layered on the gels. After electrophoresis (150 min., 5 mA/gel, 10 V/cm.) gels were rinsed with distilled water for 30 min. and scanned, using transmitted u.v. irradiation by the method of Loening (1969) (except that the *p*-dimethylaminobenzaldehyde filter was omitted). Gels were then sectioned and prepared for radioactive counting as described earlier.

Radioisotopes. Reconstituted [¹⁴C]yeast protein hydrolysate was purchased from Schwarz Biochemicals Inc., Orangeburg, N.Y., U.S.A. [³H]uridine (13 Ci/m-mole) and [³H]cytidine (30.2 Ci/m-mole) were purchased from the Radiochemical Centre, Amersham, Buckinghamshire. Carrier-free [³²P] as orthophosphate (10 to 25 Ci/mg.P) was purchased from the Australian Atomic Energy Commission, Lucas Heights, Sydney, N.S.W., Australia.

RESULTS

RNA and protein synthesis requirements during germination

The effect of proflavine on RNA and protein synthesis in *Alternaria solani*, *Aspergillus nidulans*, *Neurospora crassa* and *Peronospora tabacina* is shown in Fig. 1. Addition of proflavine was followed, 10 min. later, by either [^3H]uridine or [^{14}C]-labelled amino

Fig. 1. Effect of proflavine on RNA synthesis, protein synthesis, and germination in *Peronospora tabacina*, *Neurospora crassa*, *Aspergillus nidulans*, and *Alternaria solani*. For RNA and protein synthesis proflavine was added to conidia of *A. solani* and *P. tabacina* at the start of germination, and to *N. crassa* and *A. nidulans* conidia after 135 and 225 min. incubation, respectively. In all cases, addition of proflavine was followed 10 min. later by the addition of either [^3H]uridine or [^{14}C]-labelled amino acids. Conidia were incubated in the presence of the isotope for 15 min., except for *A. solani* where uptake was for 120 min. Incorporation was stopped by adding 0.5 ml. cold 30% (w/v) TCA and conidia were then treated as described previously (Hollomon, 1969). For germination proflavine was added to all conidia at the start of germination and the percentage germination was assessed after 4 h. at 15° for *P. tabacina*, 8 h. at 30° for *N. crassa*, 8 h. at 37° for *A. nidulans*, and 6 h. at 26° for *A. solani*. Results are presented as % of control where control represents the value obtained in the absence of proflavine. ●—●, Germination; ×—×, RNA synthesis; ▲—▲, protein synthesis.

acids. Uptake of these isotopes was stopped by adding 0.5 ml. cold 30% (w/v) trichloroacetic acid (TCA), and incorporation into TCA-insoluble material was taken as a measure of RNA and protein synthesis, respectively. As in other organisms (Waring, 1966) proflavine inhibited RNA synthesis but reduced protein synthesis only at higher concentrations. Nevertheless, proflavine had a differential effect on germination (Fig. 1). With *A. nidulans* and *N. crassa* inhibition of germination by proflavine closely

Fig. 2. The incorporation of [^3H]uridine into RNA at intervals during germination. *Neurospora crassa* and *Peronospora tabacina* conidia were labelled for 15 min. pulses with $0.4 \mu\text{Ci/ml}$ [^3H]uridine and $2.0 \mu\text{Ci/ml}$ [^3H]uridine respectively. Incorporation was stopped by addition of 0.5 ml. 30% (w/v) TCA and conidia were treated as described previously (Hollomon, 1969). $\times$ — $\times$, *Neurospora crassa*; $\circ$ — $\circ$, *Peronospora tabacina*.

paralleled its effect on RNA synthesis. However, germination in *A. solani* and *P. tabacina* was unaffected by proflavine concentrations which severely inhibited RNA synthesis, but was inhibited at higher concentrations where proflavine inhibited protein synthesis. These results suggest that in *A. solani* and *P. tabacina* RNA synthesis is not required for germination, whereas in *A. nidulans* and *N. crassa* RNA synthesis is an essential requirement for germination. Similar experiments with *Puccinia graminis* were less satisfactory. Although uredospores germinated at pro-

Fig. 3. RNA synthesis in germinating *Peronospora tabacina* conidia. For each treatment, 3.2×10^7 conidia were germinated in a medium without phosphate, in eight 15.0 cm. diameter Petri dishes each containing a final volume of 20 ml. Conidia in one dish only were labelled for 20 min. with [32 P] (10 to 12 μ Ci/ml.). After incorporation conidia from all dishes were collected together on a membrane filter either immediately or after a further 100 min. incubation in the presence of excess non-radioactive phosphate (chase). RNA was extracted and fractionated as described in the methods section. 100 μ g. RNA were applied to each gel. (a) 0 to 20 min. pulse; (b) 0 to 20 min. pulse, 100 min. chase; (c) 20 to 40 min. pulse; (d) 20 to 40 min. pulse, 100 min. chase; (e) 40 to 60 min. pulse; (f) 40 to 60 min. pulse, 100 min. chase; (g) 60 to 80 min. pulse; (h) 60 to 80 min. pulse, 100 min. chase. ---, Counts/min.[32 P]; —, absorbance 620 nm. (arbitrary scale).

flavine concentrations which inhibited RNA synthesis in the other four species, uredospores failed to incorporate [^3H]uridine into RNA before germ-tube emergence, and the effect of proflavine on RNA synthesis could not be determined.

It would seem, therefore, that differences exist between species in their requirement for RNA synthesis during germination. These differences were examined in greater detail using *Peronospora tabacina* and *Neurospora crassa*. To determine the rate of RNA synthesis prior to germ-tube emergence, conidia were labelled with 15 min. pulses of [^3H]uridine given at 15 min. intervals for *P. tabacina*, and at 30 min. intervals for *N. crassa*. Although initially low, the rate of incorporation of [^3H]uridine into *N. crassa* increased as germination proceeded (Fig. 2). However, the rate of incorporation of [^3H]uridine into *P. tabacina* conidia was not only much lower, but was fairly constant throughout.

RNA synthesis in germinating *Peronospora tabacina* conidia

Because of the low rate of incorporation, sufficient radioactivity could not be applied to gels to permit satisfactory resolution of RNA labelled during germination with [^3H]uridine. This problem was overcome, however, by labelling with [^{32}P] rather than

Table 1. *Specific activity and RNAase susceptibility of [^{32}P]-labelled RNA from germinating *Peronospora tabacina* conidia*

Details of the method used to label conidia are given in Fig. 3. Susceptibility to RNAase was determined by incubation of 100 μg . pulse-labelled RNA with 10 μg . RNAase for 2 h. at 37° and precipitation of residual radioactivity on to membrane filters with cold 5 % TCA.

Time after start of germination at which [^{32}P] pulse was given (min.)	Specific activity (counts/min./mg.RNA)		Radioactivity remaining after RNAase treatment of pulse-labelled RNA (%)
	Pulse	Chase	
0 to 20	0.49×10^5	0.36×10^5	28
20 to 40	3.42×10^5	1.12×10^5	8
40 to 60	2.21×10^5	1.25×10^5	7
60 to 80	1.94×10^5	0.51×10^5	3

[^3H]uridine. Conidia were labelled with 20 min. pulses of [^{32}P] given at 20 min. intervals during the first 80 min. of germination. In some experiments, this pulse was followed by further incubation for 100 min. in the presence of excess non-radioactive phosphate (chase). At the end of each pulse and each chase period, RNA was extracted with hot (60°) phenol to ensure extraction of the large molecular weight RNA. RNA was fractionated by polyacrylamide gel electrophoresis stained with Azure B and sectioned for radioactive counting. Any degradation occurring during extraction was monitored by the recovery of [^3H]-labelled *Neurospora* RNA which was added to the conidia at the time of grinding. Recovery of this labelled RNA varied between 60 and 80 % of that initially added. Although some degradation undoubtedly occurred, this was similar in each preparation so that comparisons between preparations were possible.

Five major RNA bands were revealed when *Peronospora tabacina* extracts were stained with Azure B (Fig. 3): a fast-migrating soluble RNA band, two ribosomal RNA bands which were coincident with the ribosomal RNA bands of *Neurospora crassa*, and two bands migrating more slowly than ribosomal RNA. During the first

180 min. of germination no changes in the position or intensity of any of these bands could be detected. Although a constant amount of RNA was extracted throughout the germination period, [^{32}P] incorporation was not constant and was least during the first 20 min. (Table 1). Furthermore, 28 % of this early [^{32}P] incorporation was ribonuclease-resistant. After electrophoresis of this early RNA some radioactivity was detected in the anode buffer, suggesting incorporation into low molecular weight compounds, such as polyphosphate or simply [^{32}P] orthophosphate carried through in RNA preparation.

Much of the [^{32}P] incorporated into RNA early in germination was into soluble RNA (Fig. 3*a*). However, when germinated in the presence of ethidium bromide (50 $\mu\text{g./ml.}$), an RNA synthesis inhibitor (Waring, 1966), 20 % of the [^{32}P] incorpora-

Fig. 4

Fig. 5

Fig. 4. Effect of ethidium bromide (50 $\mu\text{g./ml.}$) on the incorporation of [^{32}P] into RNA by germinating *Peronospora tabacina* conidia. 4.0×10^6 conidia were labelled with [^{32}P] (11 to 12 $\mu\text{Ci./ml.}$) during the first 20 min. of germination. Uptake was stopped by rapidly cooling to 0° and conidia were collected on a membrane filter together with 2.8×10^7 ungerminated conidia. RNA was extracted and fractionated as described in the Methods section, and 100 $\mu\text{g.}$ RNA were applied to the gel. —, Control; ---, ethidium bromide.

Fig. 5. Polyacrylamide gel electrophoresis of RNA from ribosomes of germinating *Peronospora tabacina* conidia. Ribosomes were extracted from conidia germinated in the presence of [^{32}P] as described in Table 3. 265 $\mu\text{g.}$ ribosomes were disrupted with 0.5 % (w/v) SDS and layered on to the gel and electrophoresed as described in the Methods section. ---, Counts/min. [^{32}P]; —, absorbance 620 nm. (arbitrary scale).

Table 2. *The effect of ethidium bromide on [^3H]cytidine and [^3H]uridine incorporation into RNA in Peronospora tabacina*

Conidia ($2.0 \times 10^5/\text{ml.}$) were incubated for 40 min. in the presence of either [^3H]cytidine (2.0 $\mu\text{Ci./ml.}$) or [^3H]uridine (2.0 $\mu\text{Ci./ml.}$). Incorporation was stopped by adding 0.5 ml cold 30 % (w/v) TCA, and conidia then treated as described by Hollomon (1969) except that the hot TCA wash was omitted.

Treatment	Disintegration/min. incorporated into RNA	
	[^3H] cytidine	[^3H]uridine
Control	12,693	5,314
Ethidium bromide (50 $\mu\text{g./ml.}$)	9,913	436
Inhibition by ethidium bromide (%)	22	92

ted into RNA during the first 20 min. of germination remained, and was almost entirely in soluble RNA (Fig. 4). Whereas [^3H]uridine incorporation early in germination was inhibited by ethidium bromide, much of the [^3H]cytidine incorporation was not (Table 2). These results, together with the observation that much of the [^{32}P] incorporated into soluble RNA was metabolically unstable and was lost during the chase incubation (Figs. 3*b*, *d*), strongly suggest that [^{32}P] was incorporated into the three terminal nucleotides (*pCpCpA*) as well as into newly synthesized soluble RNA. In addition to soluble RNA, [^{32}P] was also incorporated into heterodisperse RNA, although this was largely lost during the chase incubation (Fig. 3). Longer electrophoresis increased the separation of the ribosomal RNA bands without showing any particular association of these bands with radioactivity, which suggests that perhaps ribosomal RNA synthesis did not occur early in germination.

Table 3. *Incorporation of [^{32}P] into ribosomes during germination of *Peronospora tabacina* conidia*

Conidia (8.0×10^6) were incubated for 60 min. with carrier-free [^{32}P] ($4.9 \mu\text{Ci/ml.}$) followed by a further 100 min. incubation in the presence of excess non-radioactive phosphate. Conidia were collected on a membrane filter and augmented with 4.0×10^7 ungerminated conidia. Ribosomes were isolated by the procedure already described.

Fraction	Counts/min. incorporated by 8.0×10^6 conidia
Total incorporated	455.92×10^4
$\times 10,000 \text{ g}$ ppt. (mitochondria, nuclei)	7.50×10^4
$\times 117,000 \text{ g}$ supernatant fluid (soluble fraction)	430.84×10^4
$\times 117,000 \text{ g}$ ppt. (washed ribosomes)	3.42×10^4
Washed ribosomes after ppt. with 5 % TCA	2.26×10^4
Washed ribosomes after dialysis against 0.05 M-tris + HCl (pH 7.8) + 0.01 M-sodium EDTA	2.25×10^4

Conidia of *Peronospora tabacina* were incubated with [^{32}P] during the first 60 min. of germination followed by a further 100 min. incubation in the presence of excess non-radioactive phosphate, thus reducing incorporation into metabolically unstable heterodisperse RNA. Ribosomes were then isolated and incorporation into the different fractions is given in Table 3. Much of the [^{32}P] incorporated by the conidia was contained in the soluble fraction obtained after centrifugation for 2 h. at 117,000 g, whereas little [^{32}P] was incorporated into nuclei, mitochondria, or ribosomes. Further washing of the ribosomal pellet with KMT buffer did not remove radioactivity, yet only 66 % of this incorporation into ribosomes could be precipitated with 5 % (w/v) TCA and was presumably RNA. Dialysis for 90 min. against 0.05 M-tris + HCl (pH 7.8) containing 0.01 M-sodium EDTA resulted in a 34 % loss of radioactivity from the ribosomes. When washed ribosomes were disrupted with 0.5 % (w/v) SDS and electrophoresed, no radioactivity was associated with ribosomal RNA, which confirms that no detectable ribosomal RNA synthesis occurred in *P. tabacina* during the first 60 min. of germination (Fig. 5).

RNA synthesis in germinating Neurospora crassa conidia

Neurospora crassa conidia readily incorporated [^3H]uridine during germination (Fig. 2). As a result, conidia were labelled with 60 min. pulses of [^3H]uridine given at hourly intervals during the first 4 h. of germination. Conidia were germinated with

constant shaking at 30° in flasks containing 20 ml. of medium. Following each pulse, RNA was extracted, fractionated by polyacrylamide gel electrophoresis, and stained with Azure B. As can be seen from Fig. 6 there was little evidence of degradation and four major RNA bands were detected: two ribosomal RNA bands (28S and 18S; Rifkin, Wood & Luck, 1967); two fast-migrating bands, one being soluble RNA and

Fig. 6. RNA synthesis in germinating *Neurospora crassa* conidia. Conidia were germinated with constant shaking in five 500 ml. conical flasks each containing a final volume of 20 ml. Conidia were labelled for 60 min. with [^3H]uridine (1.25 $\mu\text{Ci/ml.}$) given at 60 min. intervals after the start of germination. After incorporation conidia were collected together on membrane filters and RNA was extracted and fractionated. 23 $\mu\text{g.}$ RNA were applied to each gel. (a) 0 to 60 min.; (b) 60 to 120 min.; (c) 120 to 180 min.; (d) 180 to 240 min.; ---, Counts/min. [^3H]; —, absorbance 620 nm (arbitrary scale).

Table 4. *Specific activity of [^3H]uridine labelled RNA from germinating *Neurospora crassa* conidia*

Details of the conditions used to label conidia are given in Fig. 6

Time after start of germination at which [^3H]uridine was given (min.)	Specific activity (counts/min./mg. RNA)
0 to 60	6.96×10^5
60 to 120	7.26×10^5
120 to 180	7.68×10^5
180 to 240	7.44×10^5

the other perhaps 7S RNA. As with *Peronospora tabacina*, changes in the position or intensity of these bands during germination were not detected. The total amount of RNA extracted increased as germination proceeded whereas the specific activity remained fairly constant (Table 4). At first [^3H]uridine was incorporated equally into

ribosomal and soluble RNA but, by the third hour, in contrast to the situation found in *P. tabacina* conidia, ribosomal RNA synthesis predominated.

DISCUSSION

Germination of spores used in these experiments was inhibited by actidione, an inhibitor of protein synthesis, indicating that protein synthesis was essential for germination (unpublished results). RNA synthesis, however, was not required for germination of *Peronospora tabacina* conidia (Hollomon, 1969), *Alternaria solani* conidia, and perhaps also *Puccinia graminis* uredospores. On the other hand, proflavine inhibited the germination of *Neurospora crassa* and *Aspergillus nidulans* conidia at concentrations which also inhibited RNA synthesis, suggesting that germination of these two species required the synthesis of RNA. Some fungal species seem, therefore, to differ in their requirements for RNA synthesis during germination.

Some RNA is, nevertheless, synthesized by germinating *Peronospora tabacina* conidia, and a comparison of this synthesis with the essential RNA synthesis in germinating *Neurospora crassa* conidia revealed further differences between the two organisms. Less [³H]uridine was incorporated into RNA by *P. tabacina* conidia than was incorporated by *N. crassa* conidia, despite a fivefold increase in the [³H]uridine concentration (Fig. 2). The RNA synthesized by *N. crassa* conidia was largely ribosomal with some soluble RNA (closely resembling the pattern of synthesis in germinating *Aspergillus oryzae* (Ono, Kimura & Yanagita, 1966; Tanaka, Ono & Yanagita, 1966) whereas in *P. tabacina* ribosomal RNA synthesis was not detected. Instead, the RNA synthesized by *P. tabacina* at the start of germination was mainly soluble RNA, although some of this may represent the rapid end-labelling of the terminal *pCpCpA* sequence. After the first 20 min., but before germ-tube emergence, unstable heterodisperse RNA was synthesized in addition to the continuing synthesis of soluble RNA. Although this heterodisperse RNA may be messenger RNA, further work is required to confirm this. Early in germination a considerable proportion of the [³²P] was incorporated into RNAase-resistant material which is, presumably, a low molecular weight compound such as polyphosphate or unincorporated [³²P]orthophosphate.

During germination of both *Peronospora tabacina* and *Neurospora crassa* conidia changes in the position or intensity of the electrophoretically separated RNA bands were not detected; thus germination seemed to involve neither the appearance nor disappearance of RNA components. Although changes were not detected it is of interest to note, in view of the lack of ribosomal RNA synthesis in *P. tabacina*, two prominent bands which migrated more slowly than ribosomal RNA, and which were possibly precursor ribosomal RNA. Similar bands were barely detectable in *N. crassa* conidia where ribosomal RNA synthesis was more active.

Failure to synthesize ribosomal RNA is not unique to germinating *Peronospora tabacina* conidia. Ribosomal RNA synthesis does not occur in cleavage embryos of sea urchins (Nemer, 1963), amphibians (Brown & Gurdon, 1964), and echiuroid worms (Gould, 1969). As in germinating *P. tabacina* conidia, these systems are undergoing rapid development and, at least in sea urchins, this development also does not require the synthesis of new RNA (Spirin, 1966). Nevertheless, some RNA is synthesized by cleaving sea-urchin embryos (Wilt, 1964; Nemer & Infante, 1966), and the pattern of this synthesis is almost identical with that found in germinating *P. tabacina*

conidia. Early in cleavage radioactivity is rapidly incorporated into soluble RNA and this incorporation is largely into the three terminal nucleotides *pCpCpA* (Glisin & Glisin, 1964; Gross, Kraemer & Malkin, 1965). In addition, there is also synthesis of heterodisperse messenger-like RNA (Wilt, 1964; Nemer & Infante, 1966).

It is essential in a study of this type that RNA should be obtained with as little degradation as possible. Extraction of RNA with cold phenol + SDS from *Peronospora tabacina* conidia disrupted by ultrasonic treatment yielded degraded preparations. Undoubtedly some of this degradation was caused by the 20 min. ultrasonic treatment needed to disrupt the conidia and which has been shown to degrade *Escherichia coli* RNA (Das, Goldstein & Lowney, 1967; Fry & Artman, 1968). For this reason conidia were broken by grinding with solid CO₂ and sand. Also, it is essential during germination to prevent the growth of bacteria which occur in *P. tabacina* conidial preparations. Fortunately, bacterial growth is inhibited by chloramphenicol (100 µg./ml.) whereas germination is not. Failure to control bacterial contamination was responsible for the earlier report (Hollomon, 1969) of ribosomal RNA synthesis in *P. tabacina* conidia which conflicts with the results presented here.

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