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Synthesis of Polyadenylic Acid-Containing Ribonucleic Acid During the Germination of *Neurospora crassa* Conidia

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Ribonucleic acid (RNA) synthesized during the first 1 h of conidial germination (15 to 20, 25 to 30, and 55 to 60 min) has been characterized by sucrose-sodium dodecyl sulfate gradient centrifugation, binding to polyuridylic acid filters, and oligo(dT)-cellulose chromatography. At all labeling periods examined, polyadenylic acid-containing RNA is synthesized and associated with polysomes. In addition, ribosomal RNA is synthesized, processed, and incorporated into polysomes. Approximately 40% of the labeled RNA sedimenting between 5 and 17*S* binds to polyuridylic acid filters. RNA which binds to oligo(dT)-cellulose displays a heterogeneous distribution in sucrose-sodium dodecyl sulfate gradients with a major, broad peak at 10–16*S*. In addition, some polyadenylic acid-containing RNA sediments beyond the 25*S* marker. Approximately 3% of the [^{3}H]adenosine in pulse-labeled polysomal RNA is in polyadenylic acid segments resistant to pancreatic and T_1 ribonucleases.

Previous work (10) has shown that during the first 30 min of spore germination in *Neurospora crassa* the percentage of polysomes increases from 3 to 75%. It has also been demonstrated that ribonucleic acid (RNA) labeled with [^{3}H]adenine is synthesized and becomes associated with polysomes during this first 30 min of germination. On the basis of sedimentation values in sucrose-sodium dodecyl sulfate (SDS) gradients this RNA was postulated to be messenger-like RNA (mRNA).

Recently many investigators have demonstrated that at least some eukaryotic mRNA's possess a polyadenylic acid [poly(A)] region at the 3′ end of the mRNA molecule. Adesnik et al. (1) reported that all HeLa cell mRNA's, with the exception of histone mRNA, contain a 3′ poly(A) region. More recent studies by Milcarek et al. (9) indicate that approximately 30% of HeLa cell mRNA's lack poly(A). Poly(A)-containing mRNA appears to be ubiquitous, being found in such diverse groups as fungi (7, 8, 15), plants (3), insects (6), sea urchins (12, 19), and a variety of cultured mammalian cells (2, 5, 9, 16). The presence of a poly(A) region on mRNA molecules allows their separation from other cellular RNAs, particularly ribosomal RNA (rRNA) and transfer RNA, which do not possess poly(A) regions. Several procedures for the isolation of poly(A)-containing RNA have been developed and include binding to polyuridylic acid [poly(U)]-impregnated filters (17), to membrane filters (Millipore Corp.) (4), to oligo(dT)-cellulose (11), and to poly(U)-Sepharose (5).

The present study was undertaken to (i) determine what percentage of *Neurospora crassa* mRNA's contain a poly(A) region, (ii) to determine the kinds of poly(A) RNA that are synthesized during the first 1 h of germination of the asexual conidia, and (iii) to determine the contribution of newly synthesized mRNA to the increase in polysomes already noted.

MATERIALS AND METHODS

Harvest and culture conditions. *N. crassa* wild-type strain 74-OR23-1A (Fungal Genetics Stock Center no. 987) was used in all experiments. Conditions of harvest and culture are those outlined previously (10).

Labeling conditions. Germinated conidia at the required stage were labeled with the appropriate isotopes. Details are given in the figure legends. Isotopes used were [8-^{3}H]adenosine (specific activity, 16.8 Ci/mmol) (ICN) and [5-^{3}H]uridine (specific activity, 27 Ci/mmol) (Amersham/Searle).

Polysome isolation. Polysomes were isolated as described previously (10).

Isolation of polysomal RNA. Polysomal RNA was extracted as described previously (10) except that polysomes were precipitated with ethanol without the addition of carrier.

Preparation and use of poly(U)-glass fiber filters. Poly(U) filters were prepared by the method of Sheldon et al. (17) except that 300 μg, rather than 150, of poly(U) was added to each filter. Labeled RNA extracted from polysomes was layered on 5 to 20%

(wt/vol) sucrose gradients made up in 0.01 M triethanolamine-0.1 M NaCl-0.001 M ethylenediaminetetraacetic acid-0.5% SDS. Gradients were centrifuged in an SW27.1 rotor at 26,000 rpm for 16 h at 20 C. Gradients were monitored continuously at 260 nm, and 0.6-ml fractions were collected. From each fraction 0.2-ml aliquots were removed. To one aliquot was added 100 μg of bovine serum albumin and trichloroacetic acid to a final concentration of 10%. Samples were stored at 5 C for 1.5 h, collected on GF/C filters, washed with 5% trichloroacetic acid and 95% ethanol, dried, and counted in a toluene-Omnifluor cocktail. To a second aliquot was added 5 ml of the poly(U)-binding buffer (0.01 M triethanolamine, pH 7.5, 0.12 M NaCl). Each 5.2-ml sample was then gravity filtered through a poly(U)-impregnated GF/C filter. Before addition of sample, each poly(U) filter was washed with 50 ml of distilled water followed by 50 ml of poly(U)-binding buffer. After the sample had been gravity filtered, the filter was washed with 20 ml of poly(U)-binding buffer followed by 20-ml washes of 5% trichloroacetic acid and 95% ethanol. Filters were dried and counted in a toluene-Omnifluor cocktail.

Oligo(dT)-cellulose chromatography. Poly(A)-containing RNA was isolated from [^{3}H]adenosine-labeled polysomal RNA by the method of Nakazato and Edmonds (11). Both the unbound and bound RNAs were precipitated with ethanol at −20 C without carrier.

Ribonuclease digestion of labeled polysomal RNA. Conidia were germinated for 2 h and then labeled with 1 mCi of [^{3}H]adenosine for 5 min. Polysomal RNA was extracted, dissolved in 0.5 ml of ribonuclease buffer (0.01 M triethanolamine [pH 7.5], 0.2 M KCl) and digested at 37 C for 30 or 60 min. Ribonuclease concentrations were: pancreatic ribonuclease (Worthington), 22 μg/ml; T_1 ribonuclease (Calbiochem), 32 μg/ml; T_2 ribonuclease (Calbiochem), 28 μg/ml (10 units/ml). The reaction was stopped by the addition of 50 μg of carrier RNA and trichloroacetic acid at a final concentration of 10%. Precipitates were collected on GF/C filters and counted in toluene-Omnifluor cocktail.

RESULTS

The RNA synthesized during the first 1 h of spore germination is depicted in Fig. 1 to 3. Figure 1 shows the RNA synthesized during the interval 10 to 15 min (15-min RNA) after the spores were placed in germination medium. Acid-precipitable radioactivity indicates that both 17 and 25S rRNA are being synthesized. In addition, a large proportion of the acid-precipitable material sediments between 4 and 17S, i.e., presumptive mRNA. The distribution of poly(A)-containing RNA synthesized during the interval 10 to 15 min after initiation of spore germination reveals a heterogeneous distribution of presumptive mRNA molecules. The majority of the mRNA molecules sediment between 5 and 17S. RNA labeled between 25 to 30 min (30-min RNA) and 55 to 60 min (60-min RNA) after the spores were inoculated show basically the same acid-precipitable and poly(U)-binding profiles as seen at 15 min (Fig. 2 and 3).

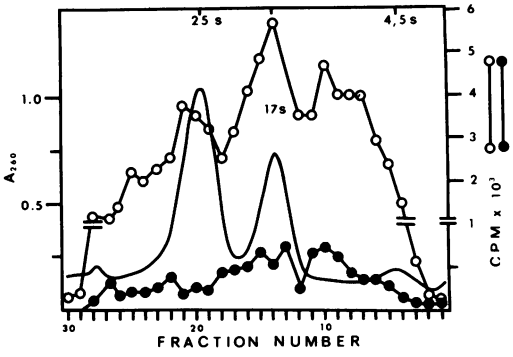

FIG. 1. *RNA synthesized during the interval 10 to 15 min (15-min RNA) after inoculation of conidia into minimal medium. One liter of minimal medium was inoculated with 6 × 10⁸ conidia per ml at 33 C. At 5 min after inoculation the conidia were concentrated 10-fold and labeled for 5 min (10 to 15 min) with 10 μCi of [³H]uridine per ml of culture medium followed by polysome isolation. Polysomal RNA was extracted and run on 5 to 20% (wt/vol) sucrose-SDS gradients. Top of the gradient is to the right. Symbols: ——, optical density at 260 nm (OD_{260}); O, [³H]uridine in acid-insoluble material; ●, [³H]uridine-labeled material binding to poly(U) filters.*

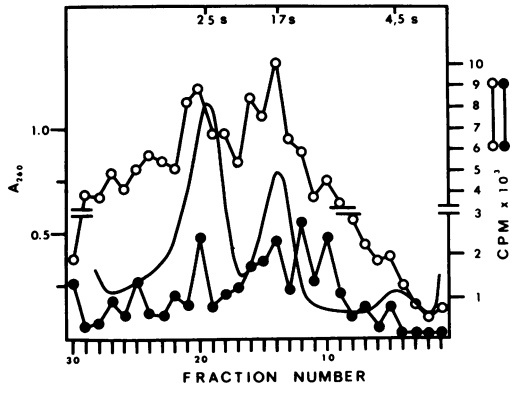

FIG. 2. *RNA synthesized during the interval 25 to 30 min (30-min RNA) after the inoculation of conidia into minimal medium. One liter of minimal medium was inoculated with 5 × 10⁸ conidia per ml at 33 C. At 20 min after inoculation the conidia were concentrated 10-fold and labeled for 5 min (25 to 30 min) with 10 μCi of [³H]uridine per ml of culture medium followed by polysome isolation. Polysomal RNA was extracted and run on 5 to 20% (wt/vol) sucrose-SDS gradient. Top of the gradient is to the right. Symbols: ——, optical density at 260 nm (OD_{260}); O, [³H]uridine in acid-insoluble material; ●, [³H]uridine-labeled material binding to poly(U) filters.*

In Fig. 4 to 6 the data are plotted as a percentage of binding to poly(U), i.e. counts per minute binding to poly(U) divided by acid-precipitable counts per minute (total counts per minute). With the exception of the 15-min RNA, the RNA molecules binding most efficiently to poly(U) filters sediment in a sucrose gradient between the 5*S* and 17*S* rRNA. The highest percentage of binding to poly(U) in the 30-min RNA is 53%, whereas in the 60-min RNA

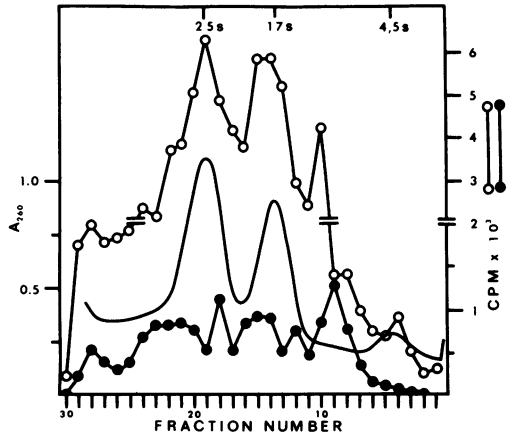

FIG. 3. *RNA synthesized during the interval 55 to 60 min (60-min RNA) after the inoculation of conidia into minimal medium. One liter of minimal medium was inoculated with 4.3 × 10^8 conidia per ml at 33 C. At 50 min after inoculation the conidia were concentrated 10-fold and labeled for 5 min (55 to 60 min) with 10 μCi of [^3H]uridine per ml of culture medium followed by polysome isolation. Polysomal RNA was extracted and run on 5 to 20% (wt/vol) sucrose-SDS gradients. Top of the gradient is to the right. Symbols: ——, optical density at 260 nm (OD_{260}); O, [^3H]uridine in acid-insoluble material; ●, [^3H]uridine-labeled material binding to poly(U) filters.*

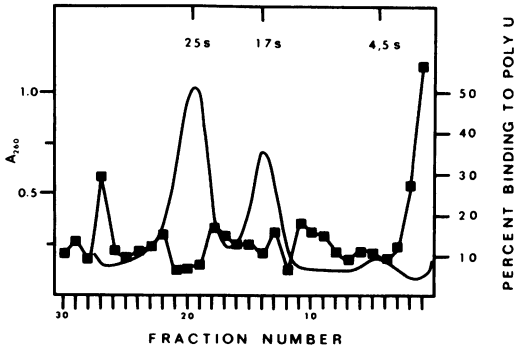

FIG. 4. *Percent binding of 15-min RNA to poly(U) filters. Percent binding for each gradient fraction shown in Fig. 1 was determined by dividing the total counts per minute binding to the poly(U) filter by the total acid-insoluble counts per minute.*

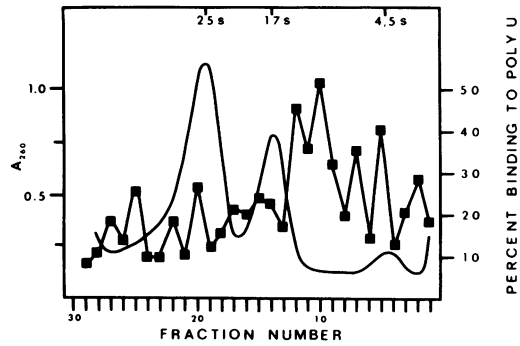

FIG. 5. *Percent binding of 30-min RNA to poly(U) filters. Percent binding for each gradient fraction shown in Fig. 2 was determined by dividing the total counts per minute binding to the poly(U) filter by the total acid-insoluble counts per minute.*

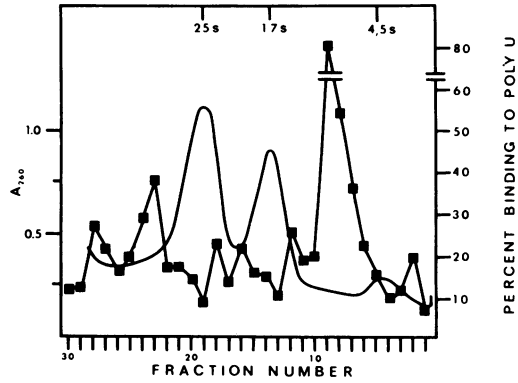

FIG. 6. *Percent binding of 60-min RNA to poly(U) filters. Percent binding for each gradient fraction in Fig. 3 was determined by dividing the total counts per minute binding to the poly(U) filter by the total acid-insoluble counts per minute.*

it is 80%. In Table 1 the percentage of binding to poly(U) for various portions of the gradient is given. The gradient is divided into three groups: presumptive mRNA (fractions 6 to 12), 17*S* and 25*S* rRNA (fractions 13 to 23), and total (fractions 1 to 30). The 15-min RNA binds to poly(U) filters at about the same level in all groups. The percentage of binding in the presumptive mRNA group is 12%. At 30 min the RNA in the mRNA fractions binds approximately three times more efficiently than at 15 min, whereas the percentage of binding in the rRNA group (13 to 23) increases slightly when compared to 15-min RNA (17.9 versus 12.0). The 60-min RNA exhibits similar binding to the 30-min RNA, with 34.4% of the radioactivity in presumptive mRNA binding to poly(U) filters. Binding of RNA in the rRNA group remains at the level seen at 30 min. The efficiency of RNA

TABLE 1. *Efficiency of RNA binding to poly(U) filters*

RNA	Group	Counts/min binding to poly(U) filters	Trichloroacetic acid-insoluble counts/min	% Binding to poly(U)
15 min	1-30	10,133	81,123	14.0
	6-12	3,797	27,369	12.5
	13-23	4,887	40,495	12.0
30 min	1-30	29,200	137,731	21.3
	6-12	8,800	24,300	36.2
	13-23	14,168	79,206	17.9
60 min	1-30	16,239	79,277	20.5
	6-12	4,711	13,705	34.4
	13-23	8,616	50,918	16.9

binding to poly(U) over the entire gradient increases from 14% at 15 min to approximately 20% thereafter.

To separate the bulk of the poly(A)-containing RNA, [^{3}H]adenosine-labeled polysomal RNA was extracted from a 1-h culture and chromatographed on oligo(dT)-cellulose. Approximately 13% of the input was bound to oligo(dT)-cellulose and eluted with buffer lacking NaCl at 60 C. Both bound and unbound RNA from the oligo(dT)-cellulose column were precipitated and centrifuged on 5 to 20% (wt/wt) sucrose-SDS gradients.

Figure 7 shows the optical density and radioactivity profile of RNA which does not bind to oligo(dT)-cellulose. The acid-precipitable radioactivity profile is basically similar to total polysomal RNA from a 1-h culture. Radioactivity is found associated with all rRNA species and some RNA species possessing sedimentation values greater than 25*S*. In addition a peak of radioactivity is found between 5*S* and 17*S* rRNA, i.e., presumptive mRNA based on sedimentation properties.

Figure 8 shows the profile for the RNA which binds to oligo(dT)-cellulose under the conditions used. The optical density profile is essentially the profile of a blank gradient, and the RNA is detected on the basis of radioactivity. The radioactivity profile is significantly different from the unbound profile, with no indication of rRNA species. The majority of the bound RNA [poly(A) containing] sediments between 5*S* and 17*S*, but a significant fraction (43%) sediments between the 17*S* rRNA and the bottom of the gradient. Fractions were collected from this region of the gradient (fractions 16 to 30) and subsequently percolated through poly(U) filters. Binding of this RNA (>17*S*) to poly(U) filters reveals a profile of peaks corresponding to those seen in the gradient (fractions 16 to 30) depicted in Fig. 4, which is the profile of unfractionated 1-h polysomal RNA. This indicates that the RNA (>17*S*) binding to poly(U) filters (Fig. 1 to 3) is poly(A) containing and not rRNA contamination.

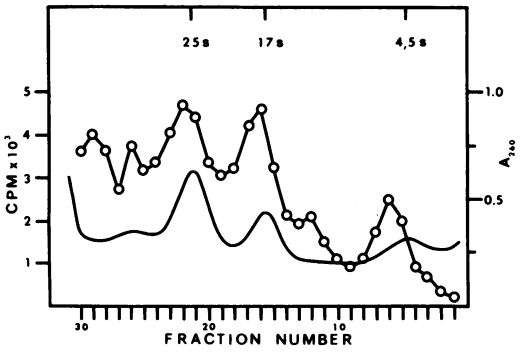

FIG. 7. *Sucrose gradient analysis of polysomal RNA which did not bind to oligo(dT)-cellulose. Two 1-liter cultures were inoculated with 5 × 10⁸ conidia per ml. At 50 min after inoculation, conidia were concentrated 10-fold and labeled for 5 min with 5 μCi of [³H]adenine per ml followed by polysome isolation. Polysomal RNA was separated into an RNA fraction which did not bind and one fraction which did bind to oligo(dT)-cellulose. The unbound RNA was analyzed on a 5 to 20% (wt/wt) sucrose-SDS gradient. Top of gradient is to the right. Symbols: ——, optical density at 260 nm (OD_{260}); O, counts per minute.*

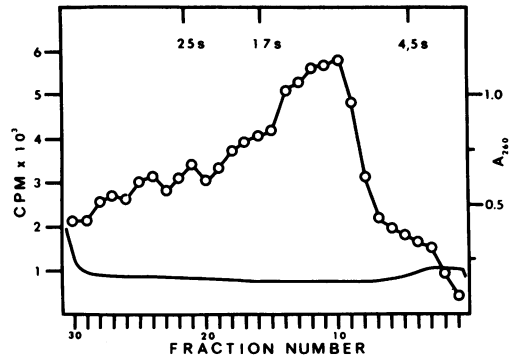

FIG. 8. *Sucrose gradient analysis of polysomal RNA which did bind to oligo(dT)-cellulose. Two 1-liter cultures were inoculated with 5 × 10⁸ conidia per ml. At 50 min after inoculation, conidia were concentrated 10-fold and labeled for 5 min with 5 μCi of [³H]adenine per ml followed by polysome isolation. Polysomal RNA was separated into an RNA fraction which did not bind and one fraction which did bind to oligo(dT)-cellulose. The bound RNA was analyzed on a 5 to 20% (wt/wt) sucrose-SDS gradient. Top of gradient is to the right. Symbols: ——, optical density at 260 nm (OD_{260}); O, counts per minute.*

To further characterize the labeled RNA, polysomal RNA was digested with ribonucleases A and T_1 or ribonucleases A, T_1, and T_2. Digestion with A and T_1 yields poly(A) segments which are resistant to digestion (18). The addition of ribonuclease T_2 results in the digestion of poly(A) segments. Table 2 gives the results of nuclease digestion of two different preparations of [^{3}H]adenosine-labeled polysomal RNA. The percentage of [^{3}H]adenosine as poly(A), i.e., radioactivity resistant to ribonucleases A and T_1 less radioactivity resistant to ribonucleases A, T_1, and T_2, ranges from 1.53 to 4.88%. These values are in close agreement with those obtained in yeast by McLaughlin et. al. (8).

DISCUSSION

The data presented confirm and extend earlier studies and demonstrate the following points. First, at all three time periods (10 to 15, 25 to 30, and 55 to 60 min) of germination studied, labeled RNA is found in ribosomes (polysomes) actively synthesizing protein. Since the incorporation of isotope into the presumptive rRNA precursor, the processing of the precursor into the functional rRNA molecules, the formation of the ribosomal subunits, and finally the formation of polysomes all occur in less than 10 min, rRNA synthesis must be a very active process in germinating conidia. In this respect conidia induced to germinate are unlike, for example, the newly fertilized sea urchin egg which does not synthesize rRNA for many hours after fertilization. Thus, although both conidia and unfertilized eggs are cells with very low metabolic activity (semidormant), the initiation of active metabolic activity results in different approaches, at least with respect to rRNA synthesis. This is probably related to the fact that *Neurospora* quite rapidly undergoes a net growth, whereas the sea urchin embryo during early development does not.

Second, presumptive mRNA, based on sedimentation values, is synthesized at all times during the first 1 h of germination. This RNA contributes to the increase in polysomes which occurs during the initial stages of germination. The data presented give further evidence that at least some of the presumed mRNA's sedimenting between 5 and 17*S* are indeed mRNA's based on the additional criterion of poly(A) content, since they bind to poly(U) filters and oligo(dT)-cellulose and are partially resistant to ribonucleases A and T_1. The profile of RNAs binding to poly(U) filters exhibits several peaks, corresponding to molecules with sedimentation values from approximately 5*S* to some with values greater than 25*S*.

In the previous study (10) RNA was pulse labeled with [^{3}H]adenine, and the possibility exists that the labeled RNA extracted from polysomes represents RNA which had preexisted in the cytoplasm and was subsequently polyadenylated after the beginning of germination. Such a process has been shown to occur in sea urchins (18). However, in this study the incorporation of [^{3}H]uridine internally in the growing transcript indicates synthesis and precludes polyadenylation of preexisting mRNA. Polyadenylation of preexisting mRNA may occur in *Neurospora* as it apparently does in the sea urchin, but these studies would not reveal it.

Assuming that the acid-precipitable material in fractions 6 to 12 is mRNA, then the poly(U), binding data for these fractions indicates that not all mRNA's contain a poly(A) region. If every mRNA possesses a poly(A)-rich region, all of the acid-precipitable radioactivity should bind to the poly(U) filters. As indicated in

TABLE 2. *Ribonuclease digestion of [^{3}H]adenosine-labeled polysomal RNA*[a]

[^{3}H]adenosine-labeled polysomal RNA (μg)	Pancreatic RNase	T_1 RNase	T_2 RNase	Reaction time (min)	Counts/min resistant to pancreatic and T_1 RNase's (%)	Counts/min resistant to pancreatic, T_1, and T_2 RNase's (%)	Counts/min as poly(A) (%)
4.5	+	+	−	30	4.94		4.88
4.5	+	+	+	30		0.26	
4.5	+	+	−	60	2.70		2.70
4.5	+	+	+	60		0.00	
2.5	+	+	−	30	2.05		1.79
2.5	+	+	+	30		0.26	
2.5	+	+	−	60	2.25		1.53
2.5	+	+	+	60		0.72	

[a] RNase, Ribonuclease.

Table 1, less than 40% of the acid-precipitable radioactivity in fractions 6 to 12 is retained on poly(U) filters. This would indicate that approximately 40% of *Neurospora* mRNA's contain a poly(A)-rich region of sufficient length to be bound to poly(U). The reliability of this figure (40%) depends on several assumptions: (i) all RNA molecules sedimenting between 5*S* and 17*S* are bona fide mRNA molecules and not degradation products of high-molecular-weight non-mRNA, (ii) polysomal RNA is not contaminated by heterogeneous nuclear RNA, (iii) all poly(A)-containing mRNA is isolated by the extraction procedures used, and (iv) all poly(A)-containing RNAs bind to poly(U) filters under the conditions employed.

Concerning these assumptions the following points are relevant. First, the optical density profiles representing essentially rRNA species do not indicate degradation, since the 2:1 ratio of 25*S* rRNA-17*S* rRNA is always obtained. This does not, however, exclude limited degradation. Second, if heterogeneous nuclear RNA was released during the disruption of conidia, heterogeneous ribonucleoprotein complexes would appear in the 10,000-rpm supernatant and would co-sediment with polysomes in sucrose gradients. To eliminate the possibility of heterogeneous ribonucleoprotein complex contamination, polysomes were treated with puromycin and recentrifuged on sucrose gradients. Less than 10% of the labeled RNA sedimented beyond the 60*S* subunit (data not shown). Since puromycin dissociates polysomes into subunits and messenger ribonucleoprotein complexes while having no effect on contaminating ribonucleoprotein complexes (13), these results indicate that at least 90% of the labeled RNA is an integral part of polysomes. Third, extraction procedures were used which are reported to yield maximal extraction of poly(A)-containing RNA (14). In addition the co-extraction of unlabeled RNA with commercially available ^{3}H-labeled poly(A) indicates that 97% of the radioactivity is recovered by the extraction procedures employed. Again, this does not eliminate the loss of the poly(A) portion of some mRNA's. Finally, under the conditions employed, 100% of ^{3}H-labeled poly(A) binds to poly(U) filters. However, since the average number of residues in the ^{3}H-labeled poly(A) is approximately 500 and since preliminary evidence indicates that the poly(A) region of *Neurospora* RNA is approximately 50 to 100 residues (unpublished data), the failure of all presumptive mRNA's to bind to poly(U) may reflect the presence of mRNA's with poly(A) regions too short to bind to poly(U). The separation of *Neurospora* polysomal RNA on oligo(dT)-cellulose which reportedly retains 90% of HeLa cell poly(A)-containing RNA apparently does not retain all of the *Neurospora* mRNA present. Upon sucrose gradient centrifugation, the unbound RNA from oligo(dT)-cellulose chromatography contains RNAs sedimenting between 5 and 17*S* (Fig. 8). This RNA is presumed to be mRNA based upon its sedimentation and the fact that unfractionated RNA sedimenting in this region of the sucrose gradient most efficiently binds to poly(U) filters. Recent reports from several laboratories indicate that 10 to 50% of mRNA's lack poly(A) (9, 12). Thus, the figure of 40% of *Neurospora* mRNA's having a poly(A) region must be considered tentative and, based upon the assumptions outlined, must be considered a minimum figure.

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LITERATURE CITED

1. Adesnik, M., M. Salditt, W. Thomas, and J E. Darnell. 1972. Evidence that all messenger RNA molecules (except histone messenger RNA) contain poly(A) sequences and that the poly(A) has a nuclear function. J. Mol. Biol. **71**:21-30.
2. Faust, C. H., H. Diggelman, and B. Mach. 1973. Isolation of poly(adenylic acid)-rich ribonucleic acid from mouse myeloma and synthesis of complementary deoxyribonucleic acid. Biochemistry **12**:925-931.
3. Higgins, T. J. V., J. F. B. Mercer, and P. B. Goodwin. 1973. Poly(A) sequences in plant polysomal RNA. Nature (London) New Biol. **246**:68-69.
4. Lee, S. Y., J. Mendecki, and G. Brawerman. 1971. A polynucleotide segment rich in adenylic acid in rapidly-labeled polyribosomal RNA component of mouse sarcoma 180 ascites cells. Proc. Natl. Acad. Sci. U.S.A. **68**:1331-1335.
5. Lindberg, V., and T. Persson. 1972. Isolation of mRNA from KB-cells by affinity chromatography on polyuridylic acid covalently linked to sepharose. Eur. J. Biochem. **31**:246-254.
6. Lizardi, P. M., R. Williamson, and D. D. Brown. 1975. The size of fibroin messenger RNA and its polyadenylic acid content. Cell **4**:199-205.
7. Loo, M. 1975. *Neurospora crassa* temperature-sensitive mutant apparently defective in protein synthesis. J. Bacteriol. **121**:286-295.
8. McLaughlin, C. S., J. R. Warner, M. Edmonds, H. Nakazato, and M. Vaughan. 1973. Polyadenylic acid sequences in yeast messenger ribonucleic acid. J. Biol. Chem. **248**:1166-1171.
9. Milcarek, C., R. Price, and S. Penman. 1974. The metabolism of a poly(A) minus mRNA fraction in HeLa cells. Cell **3**:1-10.
10. Mirkes, P. E. 1974. Polysomes, ribonucleic acid, and protein synthesis during germination of *Neurospora crassa* conidia. J. Bacteriol. **117**:196-202.

11. Nakazato, H., and M. Edmonds. 1974. Purification of messenger RNA and heterogeneous nuclear RNA containing poly(A) sequences, p. 431-443. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 29. Academic Press Inc., New York.
12. Nemer, M., M. Graham, and L. M. Dubroff. 1974. Co-existence of non-histone messenger RNA species lacking and containing polyadenylic acid in sea urchin embryos. J. Mol. Biol. **89**:435-454.
13. Penman, S., C. Vesco, and M. Penman. 1968. Localization and kinetics of formation of nuclear heterodisperse RNA, cytoplasmic heterodisperse RNA and polyribosome-associated messenger RNA in HeLa cells. J. Mol. Biol. **34**:49-69.
14. Perry, R. P., J. LaTorre, D. E. Kelley, and J. R. Greenberg. 1972. On the lability of poly(A) sequences during extraction of messenger RNA from polyribosomes. Biochim. Biophys. Acta **262**:220-226.
15. Reed, J., and E. Wintersberger. 1973. Adenylic acid-rich sequences in messenger RNA from yeast polysomes. FEBS Lett. **32**:213-217.
16. Rosenfeld, M. G., I. B. Abrass, J. Mendelsohn, B. A. Roos, R. F. Boone, and L. D. Garren. 1972. Control of transcription of RNA rich in polyadenylic acid in human lymphocytes. Proc. Natl. Acad. Sci. U.S.A. **69**:2306-2311.
17. Sheldon, R., C. Jurale, and J. Kates. 1972. Detection of polyadenylic acid sequences in viral and eukaryotic RNA. Proc. Natl. Acad. Sci. U.S.A. **69**:417-421.
18. Stoltzfus, C. M., A. J. Shatkin, and A. K. Banerjee. 1973. Absence of polyadenylic acid from reovirus messenger ribonucleic acid. J. Biol. Chem. **248**:7993-7998.
19. Wilt, F. H. 1973. Polyadenylation of maternal RNA of sea urchin eggs after fertilization. Proc. Natl. Acad. Sci. U.S.A. **70**:2345-2349.