PARTIAL PURIFICATION OF THE "Messenger RNA" OF NEUROSPORA CRASSA CONTROLLING FORMATION OF TRYPTOPHAN SYNTHETASE ENZYME.

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Received October 22, 1962

Cell-free extracts of the 74A "wild-type" strain of Neurospora crassa developed increased tryptophan synthetase enzyme activity when incubated with a mixture of amino acids, energy source, inorganic ions, and an inhibitor of ribonuclease activity. The increase in activity appeared to be due to synthesis of enzyme protein, and was paralleled by a net synthesis of total protein (Wainwright, 1959). Similar extracts of mutant strains affected at the td locus, controlling formation of tryptophan synthetase enzyme, neither contained nor developed the indole-serine condensing activity assayed (Wainwright, 1960, and unpublished experiments). However, "wild-type" enzymic activity was formed when extracts of mutants td_1 or td_2 were supplemented with crude preparations of RNA isolated from the "soluble phase" of extracts of the "wild-type" strain.

The active fraction has been partially purified, and appears to correspond to the "messenger RNA" fractions identified in other systems (e.g. Spiegelman, 1961, Brenner et al, 1961, Gros et al, 1961).

METHODS

Cell-free extracts of washed conidia were prepared as previously described (Wainwright, 1959, 1960). Batches of "wild-type" conidia for germination were grown and harvested in a similar manner.

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Young mycelium was obtained by incubating conidia in a minimal medium (Vogel, 1956) with vigorous aeration, at a concentration of ca 8 gm wet wt/l, for 6-8 hours at 30°C. It was harvested and washed by filtration on Whatman No. 1 filter paper, and blotted as dry as possible.

The standard reaction system, and procedures for the isolation and assay of tryptophan synthetase activity have been previously described (Wainwright, 1959, 1960).

RESULTS

No tryptophan synthetase activity was developed when extracts of mutant td_1 were incubated under standard conditions. However, enzyme activity was developed when the reaction mixture was supplemented with crude RNA isolated from the "soluble phase" of extracts of "wild-type" conidia according to Kirby's (1956) procedure.

Microsomal RNA isolated from the "wild-type" strain by similar procedures, as well as other types of RNA preparation, was inactive.

Isolation of "soluble phase" RNA from extracts prepared in the standard manner was tedious, and yielded very small amounts of crude material. Accordingly, other procedures for the isolation of active RNA preparations were examined. The most satisfactory procedure examined to date was direct extraction of intact young mycelium with aqueous phenol, followed by treatment with methoxyethanol, according to the method of Monier et al (1960). More recently, the second phenol treatment, and other precautions to eliminate RNA-ase contamination recommended by Apgar et al (1962) were adopted.

Yields of crude RNA (0.4-0.6 mg/gm wet wt. of conidia) were somewhat larger than those obtained with ungerminated conidia; and the period of incubation resulted in up to a 4-fold increase in starting material. In addition, the crude RNA preparations from young mycelium tended to be more homogeneous in size.

Material isolated from 40-hour mycelium (grown from small inocula) was inactive in our system.

Activity was found only in the fraction precipitated between 40 and 50% (v/v) 2-ethoxyethanol by Kirby's (1960) procedure. The proportion of the crude RNA recovered in this fraction varied markedly (30-80%) for different preparations. However, all the biologically active material was recovered in this fraction.

Our crude preparations were highly active, and as little as 3-10 μg of RNA per ml of incubation system caused maximal formation of tryptophan synthetase activity. The active materials after ethoxyethanol fractionation have invariably been maximally active at a concentration of 3 μg/ml, and inactive at 1 μg/ml (Table I). Activity in stimulating enzyme formation was totally destroyed by treatment with RNA-ase, but was unaffected by treatment with DNA-ase.

Assays for protein were negative.

TABLE I

Preparation Number	Activity of Purified "Messenger" Fraction: Tryptophan synthetase enzyme units/ml extract formed at RNA concentration, μg/ml			
	0	1	3	10
1	0	0	0.17	0.17
2	0	0	0.18	0.18
3	0	0	0.21	0.18
4	0	0	0.20	0.20

Mutant extract was incubated 1 hour with a standard reaction mixture supplemented with varying amounts of fractionated RNA.

The base compositions of 1 mgm. samples of purified fractions were determined after hydrolysis with formic acid and resolution of the hydrolysates by chromatography on paper (Wyatt and Cohen, 1953). The data are compared with the reported base composition of N. crassa DNA in Table II. Although

the base compositions of the active RNA fraction and of the DNA are very similar, it is noteworthy that there is an excess of pyrimidine over purine in the RNA.

TABLE II

Preparation Number+	Base Composition of "Messenger RNA", Moles per cent			
	Adenine	Cytosine	Guanine	Uracil
1	21.35 ± 0.3	27.34 ± 0.6	25.65 ± 0.6	25.67 ± 0.5
2	20.66 ± 0.9	27.95 ± 0.7	26.98 ± 0.6	24.64 ± 0.1
DNA*	23.0	26.6 ± 0.7	27.1 ± 1.4	23.3 ± 0.7 °

+ Values are the means for 4 analyses of Preparation 1, and 6 of Preparation 2.
* Data of Minagawa et al (1959). ° Thymine

When centrifuged in a linear gradient (3-20%) of sucrose for 8 hours, the bulk of the material appeared to have a sedimentation coefficient of ca 8S (Figure 1a). The fractionation with ethoxyethanol appeared to remove a slightly heavier component from the preparation. Extension of the period of centrifugation to 18 hours revealed the material to be markedly heterogeneous (Figure 1b).

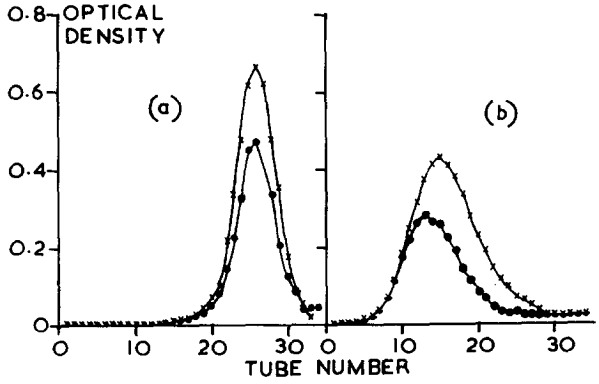

Figure 1. Sedimentation Analysis of RNA Preparations.
RNA in 0.03M Tris (pH 7.8) was layered on 4.4 ml of sucrose soln., with a linear gradient from 3 to 30% (w/v). This was centrifuged at 37,000 rpm.. Fractions were collected by piercing the lower end of the tube and collecting 10-drop fractions. To each 1 ml water was added and the optical density at 260 mμ measured.
(a) Centrifuged for 8 hours ._____. 134 μg crude RNA
(b) Centrifuged for 18 hours X_____X 187 μg fractionated RNA

The active material was also markedly heterogeneous when fractionated on ECTEOLA columns. Indeed, two distinct active fractions have been separated (McFarlane and Wainwright, in preparation).

ACKNOWLEDGEMENTS

We wish to thank Mr. N. Clark for performing the base analyses on our material.

This study was supported by grants from the Medical Research Council, The National Cancer Institute of Canada, and the Connaught Medical Research Fund.

REFERENCES

Apgar, J., Holley, R.W., and Merrill, S.H. (1962), J. Biol.Chem., 237, 798

Brenner, S., Jacob, F., and Meselson, M. (1961), Nature, 190, 576.

Gros, F., Gilbert, W., Hiatt, H.H., Attardi, G., Spahr, P.F., and Watson, J.D. (1961), Cold Spring Harbor Symp. Quant. Biol., 26, 111.

Kirby, K.S. (1956), Biochem. J., 64, 405.

Kirby, K.S. (1960), Biochem. Biophys. Acta, 40, 193.

Minagawa, T., Wagner, B., and Strauss, B. (1959), Arch. Biochem. Biophys., 80, 75.

Monier, R., Stephenson, M.L., and Zamecnik, P.C., (1960), Biochem. Biophys. Acta, 43, 1.

Spiegelman, S. (1961), Cold Spring Harbor Symp. Quant. Biol., 26, 75.

Vogel, H.J. (1956), Microbiol. Genetics Bull., 13, 42.

Wainwright, S.D. (1959), Canad. J. Biochem. Physiol., 37, 1417.

Wainwright, S.D. (1960), Nature, 185, 314.

Wyatt, G.R. and Cohen, S.S. (1953), Biochem. J., 55, 774.