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Structure of a Ribosomal 5S RNA from a Mushroom, Coprinus cinereus

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The nucleotide sequence of the 5S rRNA from a mushroom, Coprinus cinereus, was determined to be:

pAUCCACGGCCAUACGACUCUGAAAGCACCGCAUCCCGUCCGAU
CUGCGCAGUUAACCAGAGUGCCGCUCAGUUAGUACCACGGUGGGGGACCACGCGGGAAUCCUGGGUGCUGUGGUU.

This sequence is consistent with current models for the secondary structure of 5S RNAs and indicates a very high degree of sequence conservation among the most highly evolved fungi. Sequence heterogeneity was not evident in this fungus suggesting that the more highly evolved fungi may not contain the dispersed pattern of 5S rRNA genes which have been observed in intermediate fungi such as Neurospora (Selker, E.U., and Yanofsky, C. (1981) Cell 24, 819-828.

INTRODUCTION : The large subunit of all cytoplasmic ribosomes contains a 5S RNA (1) as do the ribosomes of chloroplastids (2) and even some mitochondria (3). Because of its relatively small size (approximately 120 nucleotides) and ubiquity, the 5S RNA has been an attractive model for analysis of phylogenetic relationships. Recently, with the advent of rapid gel sequencing techniques (e.g. ref. 4 and 5), a variety of sequences from many divergent origins have been determined and comparisons undertaken (e.g. ref. 6-8). One of the most striking features among the 5S RNAs from fungi is an unusual degree of sequence heterogeneity in the 5S RNA population which has been reported in certain species (9, 10). In Neurospora, this has been shown to be the result of an unusual dispersion of the 5S rRNA genes.

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While a number of sequences for the lower fungi have been determined, little attention has yet been given to the more highly evolved varieties. A somewhat unusual example of this latter type of fungus is the mushroom Coprinus cinereus which falls into the deliquescent group; fungi whose fruiting bodies mature by autolysis. In this paper, we report the nucleotide sequence for the 5S rRNA from Coprinus cinereus and compare its structure with that of other fungi. In particular, we note an apparent lack of the sequence heterogeneity which has been observed in some of the intermediate fungi (9, 10).

MATERIALS AND METHODS :

Isolation and Labeling of 5S rRNA. A dikaryotic strain of Coprinus cinereus (11) was grown with aeration at 37°C in a liquid medium containing 4 g of glucose, 10 g of malt extract and 4 g of yeast extract/liter (11). Mycelia were collected by filtration on Whatman No. 1 paper and cellular RNAs were prepared using a hot SDS-phenol extraction procedure (12). The RNA components were fractionated by electrophoresis on an 8% polyacrylamide slab gel (12) and the purified 5S rRNA was labeled at the 3' end with cytidine 3', 5'- (5'-^{32} P) bisphosphate using T_4 RNA ligase (P-L Biochemicals, Inc.) as described by Peattie (5). The labeled RNA was repurified on a 12% polyacrylamide sequencing gel (10, 12).

Determination of the Nucleotide Sequence. The nucleotide sequence was determined primarily by chemical degradation techniques, developed by Peattie (5) with the modifications described by Wildeman and Nazar (12). The 5' terminal nucleotide was confirmed by labeling the 5' end using alkaline phosphatase and polynucleotide kinase (13). The labeled RNA was digested with a mixture of pancreatic T_1 and T_2 ribonucleases and the resulting nucleotide diphosphate was determined by elecrophoresis at pH 3.5 on Whatman 3 MM paper and on DEAE paper (15).

RESULTS AND DISCUSSION : The nucleotide sequence for the Coprinus cinereus 5S rRNA (Figure 1) was determined primarily by the chemical degradation methods of Peattie (5). Except for the 5'-terminal residue, the entire

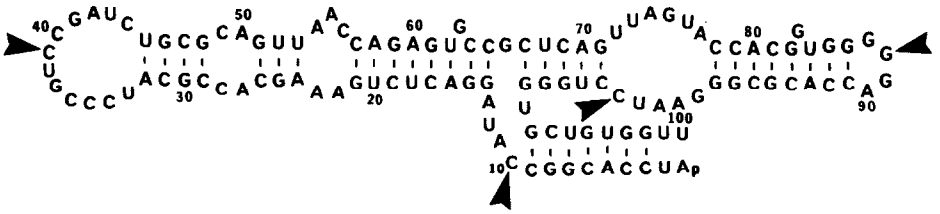

Fig. 1. Structure of the Coprinus cinereus 5S rRNA. The nucleotide sequence was determined from chemically degraded RNA as described in Figs. 2 and 3. The secondary structure is estimated according to the base-pairing scheme of Nishikawa and Takemura; the arrowheads indicate sites which were most reactive to dimethyl sulphate.

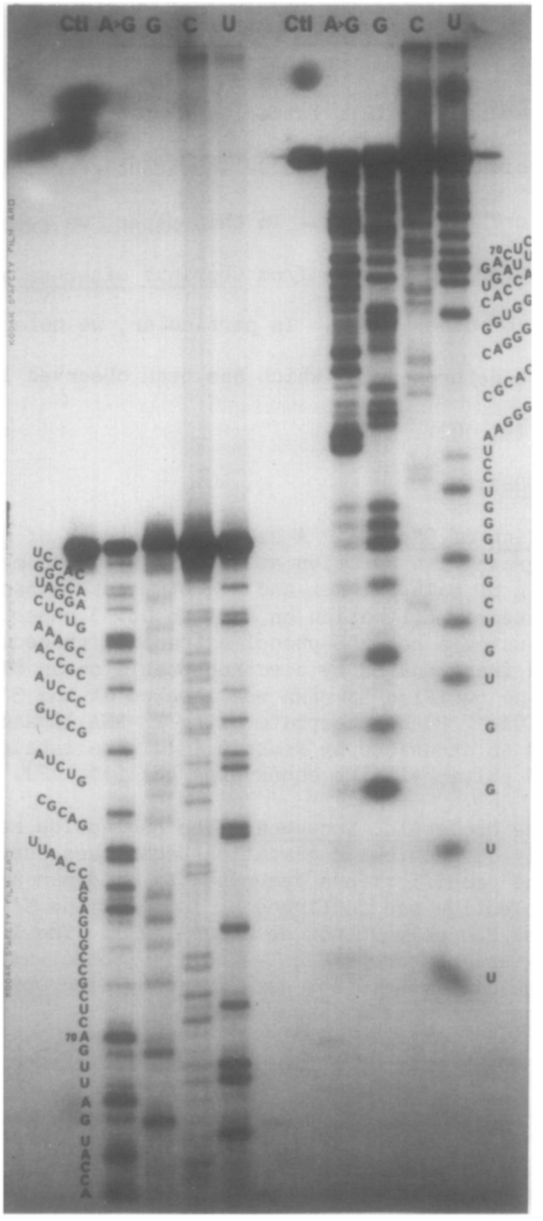

Fig. 2. Autoradiograph of 3'-end labeled Coprinus cinereus 5S rRNA degraded chemically and fractionated on a 12% polyacrylamide gel. Electrophoresis was carried out at 1050 V for 16 h (left) or 4 h (right). The sequence is indicated from the 3' to 5' end (bottom to top) in the margin; the control (Ctl) lane contained 3' end-labeled RNA treated only with aniline.

sequences could be read from sequencing gels (Figures 2 and 3). To ensure accuracy an extra long electrophoretic run (24 hours) was used to resolve the nucleotides at the 5'-end (Figure 3). No gaps were evident in any of the lanes indicating that 2'-O-methylated or pseudourydilic acid residues

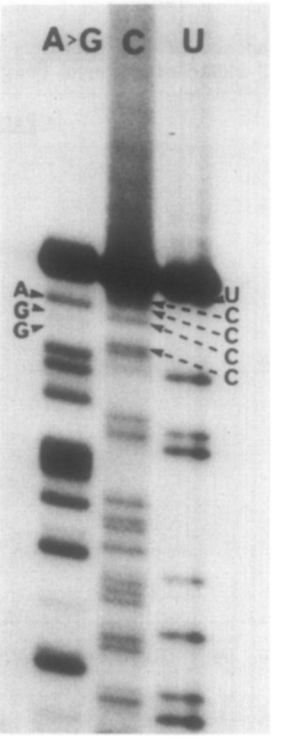

Fig. 3. Autoradiograph of 3' end labeled Coprinus cinereus 5S rRNA degraded chemically and fractionated by extended electrophoresis. Electrophoresis was carried out at 1050 V for 24 h.

were not present; of perhaps greater note was the fact that sequence heterogeneity was also not evident during either gel purification of the RNA or in the sequencing gels. Even after prolonged electrophoresis (10) alternate forms were not observed.

As shown in Figure 1, the Coprinus cinereus 5S rRNA is 118 nucleotides long with a 60% GC content. The secondary structure is completely consistent with the generalized base-pairing scheme of Nishikawa and Takemura (14); preliminary evaluations of this structure using dimethyl sulphate reactivity as a probe (15) indicate that four residues (C_{10}, C_{39}, G_{88} and C_{103}) are easily modified (results not shown).

Comparisons of the present nucleotide sequence with that of other fungi (e.g. Table 1) indicate that the sequence conservation is very high among the more evolved fungi, at least as high as has been observed with the vertebrates (6, 7). As shown in Table 1, the sequence is identical with

Table 1

Representative Comparison of the Coprinus cinereus 5S rRNA with 5S rRNAs of Diverse Origins

Organisms	Percent Sequence Homology
Schizophyllum commune	100
Tremella mesenterica	92
Ustilago violacea	66
Thermomyces lanuginosus	63
Saccharomyces cerevisiae	60
Wheat	55
Iguana	70
Drosophila	68
Chicken	68
Rat	69

The nucleotide sequences were taken from ref. 8 and 10.

that of Schizophyllum commune, a fungus in the same class but alternate family. It is 60% homologous with the 5S RNA of yeast, a much less evolved and unicellular fungus. More interesting, perhaps, is the observation that the mushroom sequence shows even greater homology (69%) with the rat 5S RNA molecule.

In view of striking differences in the distribution and heterogeneity of 5S rRNA genes between Saccharomyces and Neurospora (9), the lack of sequence heterogeneity in the 5S rRNA from a highly evolved fungi as Coprinus is surprising. This observation suggests that the heterogeneity of rRNA genes in Neurospora is not the beginning of an evolutionary pattern in higher fungi but may be restricted to a small group.

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REFERENCES :

1. Monier, R. (1974)in Ribosomes (Nomura, M., Tissieres, A., and Lengyel, P., eds.) pp. 141-168, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
2. Dyer, T.A., and Bowman, C.M. (1979) Biochem. J. 183, 595-604.
3. Cunningham, R.S., Bonen, L., Doolittle, W.F., and Gray, M.W. (1976) FEBS Lett. 69, 116-122.

4. Donis-Keller, H., Maxam, A.M., and Gilbert, W. (1977) Nucleic Acids Res. 4, 2527-2538.
5. Peattie, D.A. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 1760-1764.
6. Hori, H., and Osawa, S. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 381-385.
7. Fox, G.E., Stackebrandt, E., Hespell, R.B., Gibson, J., Maniloff, J., Dyer, T.A., Wolfe, R.S., Balch, W.E., Tanner, R.S., Magium, L.J., Zablen, L.B., Blakemore, R., Gupta, R., Bonen, L., Lewis, B.J., Stahl, D.A., Luehrsen, K.R., Chen, K.N., and Woese, C.R. (1980) Science (Wash. D.C.) 209, 457-463.
8. Walker, W.F., and Doolittle, W.F. (1982) Nature 299, 723-724.
9. Selker, E.U., and Yanofsky, C. (1981) Cell 24, 819-828.
10. Wildeman, A.G., and Nazar, R.N. (1982) J. Biol. Chem. 257, 11395-11404.
11. Lu, B.C., and Chiu, S.M. (1978) J. Cell Science 32, 21-30.
12. Wildeman, A.G., and Nazar, R.N. (1981) J. Biol. Chem. 256, 5675-5682.
13. Brownlee, G.G. (1972) in Laboratory Techniques in Biochemistry and Molecular Biology (Work, T.S., and Work, E., eds.) Vol. 3, pp. 1-265, North-Holland Amsterdam.
14. Nishikawa, K., and Takemura, S. (1977) J. Biochem. (Tokyo) 81, 995-1003. 15. Peattie, D.A., and Gilbert, W. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 4679-4682.
15. Peattie, D.A., and Gilbert, W. (1980) Proc. Natl. Acad. Sci. U.S.A.77, 4679-4682.