DISAGGREGATION OF GIANT "DNA-LIKE RNA" OF YEAST BY DENATURATION IN THE PRESENCE OF FORMALDEHYDE.

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Summary

In yeast, cycloheximide causes the accumulation of heterogeneous RNA, most of which is high molecular weight RNA sedimenting faster than the 26S ribosomal RNA component, which has a molecular weight of 1.3×10^6. Heating of this heterogeneous RNA in the presence of formaldehyde shows that this RNA is an aggregate of molecules that have molecular weights ranging from about 0.2×10^6 to 1.4×10^6.

In previous communications we have described that yeast cells and protoplasts incubated in the presence of cycloheximide accumulate a heterogeneous, rapidly sedimenting RNA (S>26) with a base composition resembling the base composition of yeast DNA (1). Later studies showed that this RNA remains in the organic phase upon phenol deproteinization of protoplasts in the absence of sodium lauryl sulphate (2). Recent studies have shown that this RNA hybridizes very efficiently with yeast DNA (3). Similar rapidly sedimenting, heterogeneous RNA species have been detected in cell nuclei of several higher organisms (4-9). It has been known for some time (10) that different RNA species like messenger RNA and ribosomal RNA can interact and form aggregates, an interaction which may seriously affect the sedimentation behaviour of the RNA. Therefore it was considered important to investigate whether the giant RNA in yeast consisted of aggregates or of

truly continuous polynucleotide molecules.

The distinction between these two possibilities usually arises from experiments involving variations of the ionic composition of the solvent in which the sedimentation studies are carried out (6,8,10). It might be, however, that the relatively mild variations in the solvent composition employed in such studies were not capable of detecting certain types of aggregation, and that more forceful methods would have to be employed in order to detect the composite nature of the RNA molecules under study.

This paper describes the results of experiments in which high molecular weight yeast RNA was heated in the presence of formaldehyde (HCHO). The results show that the heterogeneous, rapidly sedimenting RNA formed in the presence of cycloheximide is an aggregate of molecules sedimenting after formaldehyde treatment with a mean value of about 11S.

Methods

The growth of Saccharomyces carlsbergensis N.C.T.C. 74, the conversion of the cells into protoplasts, as well as the preparation of high molecular weight RNA with sodium lauryl sulphate and phenol have been described in an earlier publication (1). Incorporation of radioactive precursors, ^{14}C-adenine or ^{3}H-uracil, into RNA was carried out as described earlier except for the replacement of the mannitol by 20 percent sorbitol. Ribosomal RNA and its precursors were labelled selectively with ^{14}C-methyl-methionine. Unlabelled adenine (100 ug per ml) was added to suppress labelling of the purine skeleton. RNA was incubated with formaldehyde with some modification of the procedure described by Boedtker (11). 300 to 500 ug of high molecular weight RNA were incubated in 0.4 ml of 7.5 percent HCHO in 0.1 M potassium phosphate buffer pH 7.3 for 15 min at 63°. The samples

were rapidly cooled in ice water and put on 15-33 percent sucrose gradients prepared as described before (2). Gradients were spun for 15-20 hrs at 37,000 rev per min in the SB 283 rotor of an International Preparative Ultracentrifuge Model B-60 at 2°.

Results

The newly synthesized RNA isolated from yeast protoplasts incubated in the presence of cycloheximide sediments in a sucrose gradient as heterogeneous material with no definite mean sedimentation constant. Most of this RNA has sedimentation values well above those of the metabolically stable high molecular weight 26S and 17S ribosomal RNA species (1), (Fig. 1a). The latter are indicated by the optical density pattern at 260 mu, and the heterogeneous RNA synthesized in the presence of cycloheximide is indicated by the radioactivity pattern.

Heating the samples of RNA with formaldehyde prior to the density gradient centrifugation produces considerable changes in the sedimentation behavior of the different components (Fig. 1b). In agreement with Boedtker (11) our results show that the reaction with HCHO reduces the sedimentation rate of the high

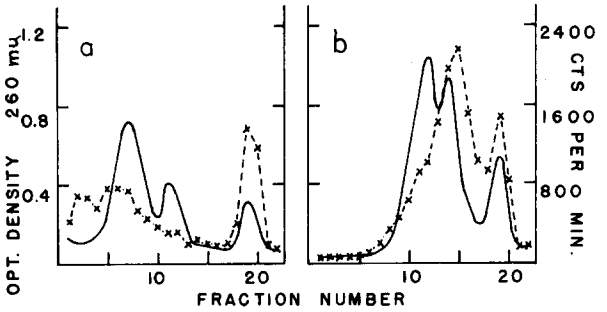

Fig. 1. Effect of denaturation in the presence of formaldehyde on the sedimentation behaviour of yeast RNA. Yeast protoplasts were incubated with ^{14}C-labelled adenine in the presence of cycloheximide for 60 min. Total RNA was isolated and centrifuged on a sucrose gradient before (a) and after heating with HCHO (b).

molecular weight ribosomal RNA components from about 26S and 17S to about 15S and 12S. This is a result of the more extended configuration of these macromolecules due to the elimination of hydrogen bonding by HCHO as a contribution to the forces determining the secondary structure of these polynucleotides. However, a far more dramatic change is observed in the sedimentation behaviour of the heterogeneous RNA accumulating in the presence of cycloheximide. The reaction with HCHO converts this RNA into material of considerably less polydispersity and lower molecular weight with a main peak sedimenting at about 11S, slightly less than the sedimentation value of the smaller of the two ribosomal RNA components.

The reduction in molecular size might be brought about by the disaggregation of RNA complexes or by breakage of phosphate-ribose bonds of truly high molecular weight RNA. The latter possibility is considered unlikely for several reasons. In the first place no degradation of the stable ribosomal RNA components occurs as is shown by the lack of an increase in the relative amount of low molecular weight material in the gradients. Furthermore, Fig. 2, a and b, shows that HCHO treatment affects the rate of sedimentation of the ^{14}C-methyl ribosomal RNA precursor and the mature ribosomal RNA species to the same relative extent. This demonstrates that RNA molecules of molecular weight over 2.5×10^6 can withstand heating with HCHO without detectable degradation. Therefore we conclude that the heterogeneously sedimenting RNA consists of aggregates of molecules with an average molecular weight of about 600,000, somewhat less than the molecular weight of the smaller of the two high molecular weight ribosomal RNA species which is assumed to have a molecular weight of 0.7×10^6 (12). The aggregate described here must be more stable than the

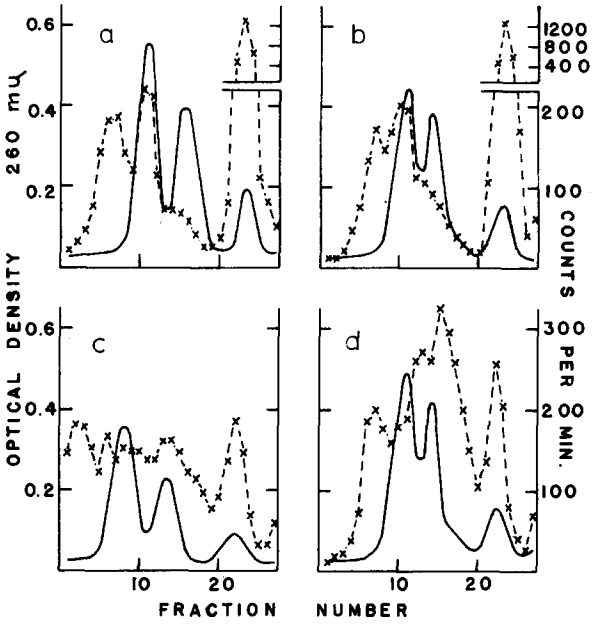

Fig. 2. Effect of denaturation in the presence of formaldehyde on the sedimentation behaviour of yeast RNA pulse-labelled with ^{14}C-methyl-methionine or ^{14}C-adenine. Yeast protoplasts were incubated for 2 min with ^{14}C-methyl-methionine (a,b), or with ^{14}C-adenine (c,d). Total RNA, before (a,c) or after (b,d) heating with HCHO were centrifuged on sucrose gradients. Gradients a and c were centrifuged for 15 hrs at 37,000 rpm. Gradients b and d were centrifuged for 20 hrs at 40,000 rpm.

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aggregates between rapidly labelled RNA and ribosomal RNA of *E. coli* described by Hayes, Hayes and Guerin (10). We, like others, in the case of nuclear RNA (6), have never observed considerable disaggregation of the heterogeneous RNA upon lowering the ionic concentration in the gradient to 0.001 M EDTA or heating to 55° in 0.1 M NaCl. Heating to 63° without the addition of HCHO causes some disaggregation (2), however not as complete as in the presence of HCHO.

That the aggregated RNA represents apparently a normal constituent of the cell and not just the result of the incubation with cycloheximide can be concluded from the observation that ^{14}C-adenine pulse-labelled RNA partially disaggregates also upon

incubation with HCHO (Fig. 2, c and d). The nondisaggregated fraction sedimenting faster than the 26S RNA represents the ribosomal precursor mentioned before.

Most likely the aggregates involve RNA-RNA interaction since DNAse and pronase were without effect and no protein could be detected in high molecular weight RNA in experiments in which the cells were prelabelled with ^{14}C-lysine prior to the incubation with cycloheximide, a method which would allow the detection of the presence of minute (less than 0.01 percent) quantities of protein.

Discussion

The results presented above show that the giant heterogeneous RNA synthesized in the presence of cycloheximide is in fact an aggregate of RNA subunits with molecular weights ranging from a few hundred thousand to about one million. The fact that disaggregation occurs after heating in the presence of HCHO suggests strongly that these subunits are kept together by hydrogen bonds.

The function of this RNA is not known. The fact that it accumulates when translation is inhibited by cycloheximide and the observation that polysomes continue to be formed (1) as well as the finding that it is a transcript of a considerable fraction of the DNA (3), suggest that at least part is messenger RNA. With this respect, it is interesting to remark that after HCHO treatment the molecular weight range of RNA accumulating in the presence of cycloheximide (200,000 to 1,400,000) is in accordance with the molecular weight of messenger RNA necessary to code for polypeptide chains with a molecular weight varying between 20,000 and 140,000, the molecular weight of polypeptides most common in nature.

The RNA described here shows also a strong resemblance with

the heterogeneous RNA of unknown function of the nucleolochromosomal apparatus of higher cells described by Georgiev (7) and others (4,5,6,8,9,13,14).

In view of our experience with similar heterogeneous yeast RNA described in this paper, it might be interesting to subject this nuclear material also to denaturation in the presence of formaldehyde in order to establish whether this RNA is an aggregate rather than a truly high molecular weight RNA.

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References

1. de Kloet, S. R. Biochem. J. 99, 566 (1966).
2. Mayo, V. S., B. A. G. Andrean, S. R. de Kloet. Biochim. Biophys. Acta 169, 297 (1968).
3. de Kloet, S. R. Arch. Biochem. and Biophys. 136, 402 (1970).
4. Scherrer, K., L. Marcaud, F. Zajdela, J. M. London, F. Gros. Proc. Nat. Acad. Sci. U. S. 56, 1571 (1966).
5. Willems, M., H. A. Musilova, R. A. Malt. Proc. Nat. Acad. Sci. U. S. 62, 1189 (1969).
6. Stevenin, J., P. Mandel, M. Jacob. Proc. Nat. Acad. Sci. U. S. 62, 490 (1969).
7. Georgiev, G. P. Prog. Nucl. Acid Res. Molec. Biol. 6, 259 (1967).
8. Attardi, G., H. Parnas, M. I. H. Hwang, B. Attardi. J. Mol. Biol. 20, 145 (1966).
9. Roberts, W. K., V. E. Quinlivan. Biochemistry 8, 288 (1969).
10. Hayes, D. H., F. Hayes, M. F. Guerin. J. Mol. Biol. 18, 499 (1966).
11. Boedtker, H. J. Mol. Biol. 35, 61 (1968).
12. Loening, U. E. J. Mol. Biol. 38, 355 (1968).
13. Church, R., B. J. McCarthy. Proc. Nat. Acad. Sci. U. S. 58, 1548 (1967).
14. Britten, R. J., E. H. Davidson. Science 165, 349 (1969).