ISOLATION OF A DNA-RNA COMPLEX FROM *EUGLENA GRACILIS*

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1. Introduction

Naturally-occurring DNA-RNA complexes have been reported in bacteria [1, 2], *Neurospora* [3], *Chlorella* [4], *Drosophila* [5], higher plants [6], and liver [7]. A peak of RNA intermediate between rRNA and tRNA in *Euglena gracilis* was claimed to be a DNA-RNA complex [8] on the basis that a similar peak was found to be a DNA-RNA complex in higher plants [6]. However, no experimental evidence for such a complex was presented in the case of *Euglena* [8]. In this report, we present data which demonstrate that a DNA-RNA complex does exist in *E. gracilis*.

2. Methods

Cultures of chloroplast-free *E. gracilis* (strain SM-L1, streptomycin-bleached) were grown in the dark, harvested and disrupted in a French pressure cell [9]. Total RNA was extracted with phenol and purified [9]. The purified total RNA was extracted 4–5 times with cold 1 M NaCl. The saline-soluble fractions were pooled together (4–5 ml total containing 4 mg/ml of 260 nm-absorbing material) and were mixed with twice their volume of isopropanol (Mallinckrodt, reagent grade, prechilled to -20°). A fibrous DNA-like precipitate separated and could be "spooled-out" on a glass rod. The spooled precipitate was dissolved in about 1 ml of 5 mM tris (pH 7.4), precipitated again with isopropanol, dried with nitrogen gas, and dissolved again in tris at a concentration of 2 mg/ml (1 A_{260} unit = 40 μg).

The spooled precipitate was electrophoresed, as described in the legend to fig. 1, on a polyacrylamide gel prepared according to Bishop et al. [10]. The spooled precipitate was also treated with heat, DNAase and RNAase as described in the legend to fig. 2.

Protein, DNA and RNA were determined by the methods of Lowry et al. [11], Burton [12] and Ceriotti [13], respectively.

3. Results and discussion

Total RNA contained about 5% DNA and 2.6% protein (table 1). Repeated extractions of the total RNA with phenol, chloroform and sodium tri-isopropylnaphthalene sulfonate [9] did not lower the DNA or protein content. About 30% of the RNA and all the DNA and protein was solubilized by 1 M NaCl. The composition of the precipitate spooled from the saline-soluble fraction was 53% RNA, 32% DNA and 15% protein.

When electrophoresed on polyacrylamide gels, the spooled fraction ran as a single peak and stained positively with diphenylamine (fig. 1). Heat denaturation, which breaks hydrogen bonds, caused the spooled fraction to appear as two peaks (fig. 2a). The spooled fraction (fig. 1) was resistant to RNAase and showed hyperchromicity when treated with DNAase [14]. The two peaks resulting from heat denaturation (fig. 2a), however, were attacked by these enzymes, but the diphenylamine-negative peak was attacked only by RNAase (fig. 2b) and the diphenylamine-positive peak was attacked only by DNAase (fig. 2c).

It is concluded from these results that the spooled fraction contains DNA and RNA in the form of a complex bound through hydrogen bonds, and that the complex also contains some protein. The manner in which the protein is associated with the complex is, however, not yet known.

Table 1
Composition of RNA fractions.

Fraction	RNA	DNA	Protein
	(mg/100 mg)		
Total RNA	92.3	5.1	2.6
Saline-insoluble	>99.2	negligible	negligible
Saline-soluble	76.0	17.0	7.0
Spooled fraction	53.0	32.0	15.0

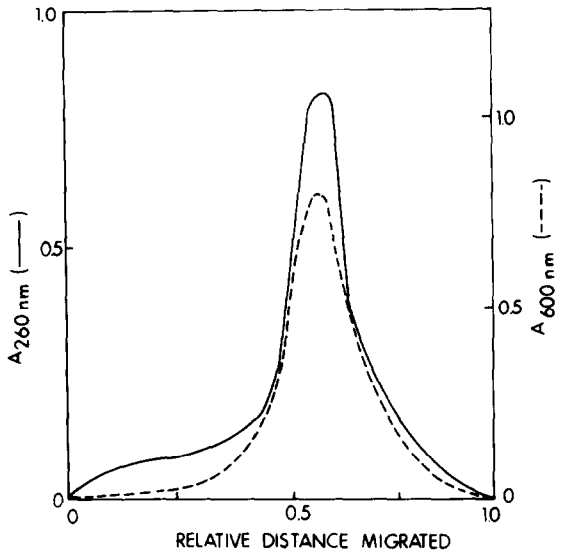

Fig. 1. Polyacrylamide gel electrophoresis and diphenylamine-staining of the "spooled fraction". Ten μg of the "spooled fraction" in 5 mM tris buffer (pH 7.4) and 0.3 M sucrose were electrophoresed on a 2.8% polyacrylamide gel which was then scanned at 260 nm. The gel was then stained with diphenylamine reagent [12] and scanned at 600 nm.

Recent experiments show that the DNA-RNA complex reported here has template activity as tested by an *Escherichia coli* cell-free protein synthesis system [14]. Experiments are underway to determine the cellular location and the specific function of this complex in *Euglena*.

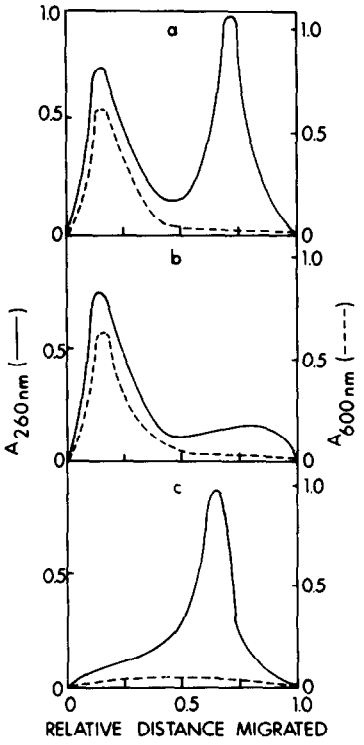

Fig. 2. Effects of heat and of RNAase and DNAase on the heat-denatured "spooled fraction".

(a) "Spooled fraction", 2 mg per ml of tris-sucrose (see fig. 1) was heated at 100° for 12 min and then quick-chilled in ice.

(b) Heat-denatured "spooled fraction" was treated with 1 μg of Worthington bovine-pancreatic RNAase (DNAase-free) at 2–4° for 20 min.

(c) Heat-denatured "spooled fraction" was treated with 50 μg of Worthington bovine-pancreatic DNAase (RNAase-free) in 0.05 M Mg^{2+} at 2–4° for 20 min and dialysed at 4° for 18 hr against water. All samples were electrophoresed and stained as in fig. 1.

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References

[1] S.Spiegelman, B.H.Hall and R.Storch, Proc. Natl. Acad. Sci. U.S. 47 (1961) 1135.

[2] V.S.Tongur, N.S.Wladthchenskaya and W.M.Kotchkina, J. Mol. Biol. 33 (1968) 451.
[3] H.M.Schulman and D.M.Bonner, Proc. Natl. Acad. Sci. U.S. 48 (1962) 53.
[4] G.Richter and H.Senger, Biochim. Biophys. Acta 95 (1965) 362.
[5] C.G.Mead, J. Biol. Chem. 239 (1964) 550.
[6] J.H.Cherry, Science 146 (1964) 1066.
[7] A.O.Pogo, J. Cell Biol. 39 (1968) 106a.
[8] A.Gnanam and J.S.Kahn, Biochim. Biophys. Acta 142 (1967) 475.
[9] K.E.Schuit and D.E.Buetow, Biochim. Biophys. Acta 166 (1968) 702.
[10] D.H.L.Bishop, J.R.Claybrook and S. Spiegelman, J. Mol. Biol. 26 (1967) 373.
[11] O.H.Lowry, N.J.Rosebrough, A.L.Farr and R.J.Randall, J. Biol. Chem. 193 (1951) 265.
[12] K.Burton, Biochem. J. 62 (1956) 315.
[13] G.Geriotti, J. Biol. Chem. 214 (1955) 59.
[14] N.G.Avadhani and D.E.Buetow, J. Cell Biol. 43 (1969) 8a.