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HYBRIDIZATION BETWEEN 5S rRNA AND 18S rRNA FROM BARLEY EMBRYOS AND MOUSE SARCOMA 180 ASCITES CELLS

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SUMMARY

Barley embryo 5S rRNA hybridizes efficiently with barley embryo 18S rRNA but not with 26S rRNA. Mouse sarcoma 5S rRNA also selectively hybridizes, to a smaller extent, with mouse sarcoma 18S rRNA. The barley embryo 5S-18S rRNA complex has a sharp melting profile and a "Tm" of ca. 59° in 0.1 M NaCl. The mouse sarcoma 5S-18S rRNA complex has a broader transition breadth and a "Tm" of ca 52°. The conditions used for hybridization lead to very specific reconstitution of the "natural" complex between 5.8S and 26S-28S rRNA since both the in vivo and in vitro complexes between 5.8S and 26S-28S rRNA from barley embryos and mouse sarcoma have equally sharp melting profiles and a "Tm" of ca. 52° in 0.1 M NaCl.

INDRODUCTION

One of the most important events in protein synthesis is the reversible association of ribosomal subunits. Little is known about the mechanism of this process in eukaryotes. Under conditions that lead to the in vitro reconstitution of the "natural" complex that exists between wheat embryo 5.8S and 26S rRNA, wheat embryo 5S rRNA forms an efficient and selective base-paired complex with 18S rRNA [1-3]. Because the 5S and 18S RNA are present in the 60S and 40S ribosomal subunits, respectively, it has been suggested [1] that hybridization between 5S and 18S rRNA might be involved in the reversible association of ribosomal subunits. In this context it is important to know whether specific hybridization between 5S and 18S rRNA also occurs in organisms other than wheat embryo. This report describes the formation and some properties of in vitro hybrids between 5S and 18S rRNA from barley embryos and mouse sarcoma 180 Ascites cells.

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MATERIALS AND METHODS

The materials and methods used for the preparation and purification of undegraded and electrophoretically homogenous cytoplasmic RNA species from barley embryos (Hordeum clipper) and mouse sarcoma 180 Ascites cells have been described in detail elsewhere [4]. Barley embryos were labelled as described for wheat embryo [2], and mouse sarcoma cells were labelled for 16 hours in a medium described by Lee et al. [5], but also containing 30 mM HEPES (Sigma), and 1 mCi each of [^{3}H]-labelled adenosine, guanosine, cytidine and uridine (Amersham) per 100 ml of medium containing ca. 10^9 cells.

Hybridization was carried out as described by Azad and Lane [1,2]. Reaction mixtures contained equimolar amounts of unlabelled 18S or 26S-28S rRNA and [^{3}H]-labelled 5S or 5.8S rRNA at a concentration of 1 mg/ml, unless otherwise mentioned. The reaction mixture in 0.3 M NaCl was heated at 60° for 5 min. and then cooled to 0°, and aliquots analyzed by polyacrylamide gel electrophoresis. The extent of hybridization was monitored by examining the degree to which the annealing procedure caused radioactivity to be transferred from the region of the gel occupied by freely migrating [^{3}H] 5S or [^{3}H] 5.8S RNA to the region occupied by unlabelled 18S or 26S-28S RNA.

The melting profiles of the complexes were obtained as described earlier [2]. The complexes were purified by sucrose density gradient centrifugation, and then washed successively (3 x each) with 67% ethanol, absolute ethanol, and anhydrous diethyl ether, and finally dissolved in 0.1 M NaCl. Aliquots were heated at a series of increasing temperatures for 5 min. at each temperature, and rapidly cooled in ice. They were then subjected to electrophoresis in 7.5% gels in which the undissociated hybrids remained at the origin. The percentage dissociation was determined by measuring the proportion of the total radioactivity present in the region of the gels occupied by freely migrating 5S or 5.8S rRNA.

RESULTS AND DISCUSSION

Initial hybridization experiments were carried out between unlabelled 18S

rRNA and unfractionated [^{3}H] sRNA, which contain 5S rRNA and tRNA. When a reaction mixture containing barley embryo [^{3}H] sRNA and unlabelled 18S rRNA was subjected to hybridization condition and then analyzed by 2.5% polyacrylamide gel electrophoresis, a radioactive peak appeared in the region of the gel occupied by unlabelled 18S rRNA (Fig. 1a). A radioactive 18S peak was also seen when a reaction mixture containing mouse sarcoma unlabelled 18S rRNA and [^{3}H] sRNA was analyzed by 2.5% polyacrylamide gel electrophoresis (Fig. 1b). Since radioactive 18S peaks were not seen when the reaction mixtures contained [^{3}H] sRNA alone (Fig. 1a and 1b), these results would suggest that, in case of both barley embryo and mouse sarcoma, a portion of the [^{3}H] sRNA complexes with unlabelled 18S rRNA. The reaction mixtures were then analyzed by 7.5% polyacrylamide gel electrophoresis. When the reaction mixtures contained [^{3}H] sRNA alone, two radioactive peaks corresponding to 5S rRNA and tRNA could be seen, but when unlabelled 18S rRNA was also present radioactivity in the 5S peak disappeared completely in case of barley embryo (Fig. 1c), and was considerably diminished in case of mouse sarcoma (Fig. 1d), and in both cases there was a corresponding increase in radioactivity at the origin, where the unlabelled 18S RNA remains in 7.5% gels. These results suggest that, in case of both barley embryos and mouse sarcoma, 5S rRNA complexes with 18S rRNA.

To confirm the above results, hybridization was carried out between equimolar amounts of purified [^{3}H] 5S rRNA and unlabelled 18S rRNA, and the reaction mixtures were analyzed by 2.5% polacrylamide gel electrophoresis. There was efficient complexing between barley embryo [^{3}H] 5S and unlabelled 18S rRNA, as shown by the transfer of radioactivity from the region occupied by freely migrating [^{3}H] 5S rRNA to the region occupied by unlabelled 18S rRNA (Fig. 2a). Under these same conditions there was no hybridization between barley embryo 5S and 26S rRNA, and between barley embryo tRNA and 18S rRNA (data not shown). Thus, barley embryo 5S rRNA complexes efficiently and selectively with barley embryo 18S rRNA. Mouse sarcoma [^{3}H] 5S rRNA complexed to a smaller extent with mouse sarcoma 18S rRNA (Fig. 2b) under conditions where there was very little

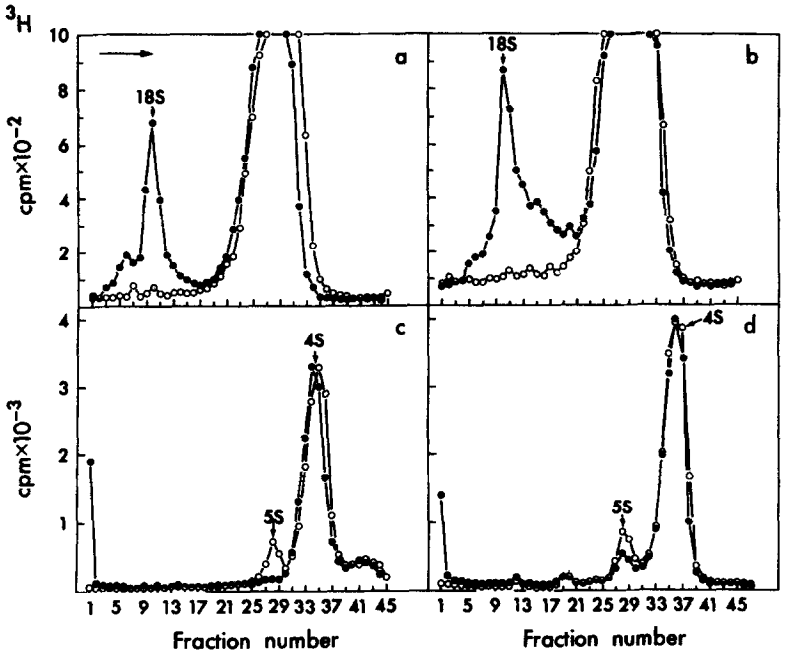

Fig. 1. Hybridization was carried out between barley embryo [^{3}H] sRNA and unlabelled 18S rRNA (Fig. 1a and 1c), and between mouse sarcoma [^{3}H] sRNA and unlabelled 18S rRNA (Fig. 1b and 1d). Reaction mixtures, containing 0.75 A_{260} unit of barley embryo or mouse sarcoma [^{3}H] sRNA and 2.0 A_{260} units of barley embryo or mouse sarcoma unlabelled 18S rRNA in 150 µl of 0.3 M NaCl, were heated at 60° for 5 minutes, cooled in ice and then analyzed by either 2.5% polyacrylamide gel electrophoresis (Fig. 1a and 1b) or 7.5% polyacrylamide gel electrophoresis (Fig. 1c and 1d). The open circles show the radioactive profiles when the reaction mixtures contained [^{3}H] sRNA alone, and the closed circles show the radioactive profile when the reaction mixtures contained [^{3}H] sRNA and unlabelled 18S rRNA.

complex-formation between mouse sarcoma 5S and 28S rRNA, and between mouse sarcoma tRNA and 18S rRNA. Thus, the complexing between mouse sarcoma 5S and 18S rRNA is selective, but much less efficient than that between barley embryo 5S and 18S rRNA. As had been found in the case of wheat embryo [2], there was quantitative reconstitution of the "natural" complex between barley embryo 5.8S and 26S rRNA, but when the reaction mixture contained equimolar amounts of mouse sarcoma [^{3}H] 5.8S and unlabelled 18S rRNA, only a portion of the 5.8S rRNA hybridized with 28S rRNA (Fig. 2c). Thus, both the 5S-18S and 5.8S-28S rRNA complexes in mouse sarcoma are formed less efficiently than the corresponding hybrids in barley and wheat embryo. The efficiency of hybridization

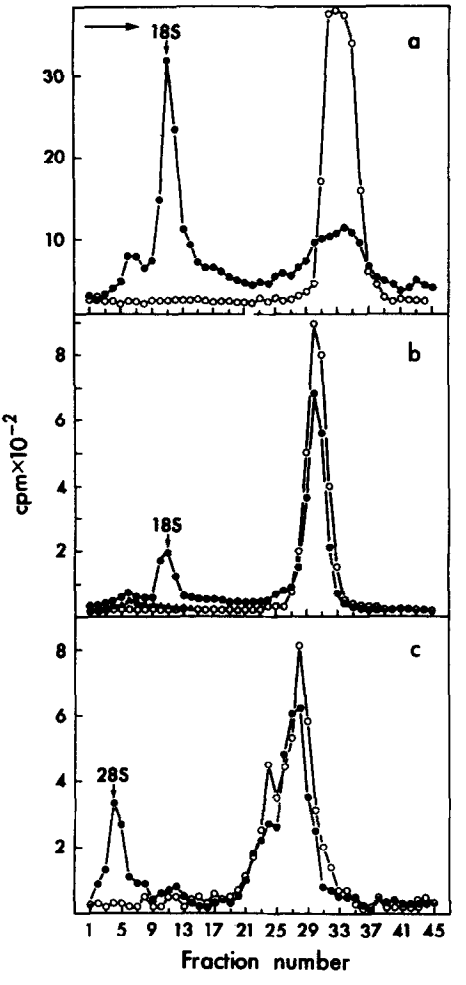

Fig. 2. These radioactive profiles in 2.5% polyacrylamide gels show the hybridization between barley embryo [^{3}H] 5S and unlabelled 18S rRNA (Fig. 2a); between mouse sarcoma [^{3}H] 5S and unlabelled 18S or 28S rRNA (Fig. 2b); and between mouse sarcoma [^{3}H] 5.8S and unlabelled 28S rRNA (Fig. 2c). The open circles show the profiles when the reaction mixtures contained only [^{3}H] 5S or [^{3}H] 5.8S rRNA; the closed circles show the profiles when the reaction mixtures also contained unlabelled 18S or 28S rRNA; the triangles in Fig. 2b show the hybridization between mouse sarcoma [^{3}H] 5S and unlabelled 28S rRNA. Reaction mixtures, containing 0.1 A_{260} unit of [^{3}H] 5S or [^{3}H] 5.8S rRNA, and 2.0 A_{260} units of unlabelled 18S rRNA or 3.0 A_{260} units of unlabelled 28S rRNA, were processed as described in Methods. The small radioactive peaks migrating behind 18S rRNA in Fig. 2a and 2b are due to aggregation of 18S rRNA that occurs when it is heated in 0.3 M NaCl.

between 5.8S and 28S rRNA is also low in sea urchin embryos [6] and rabbit reticulocytes [7].

The barley embryo and mouse sarcoma 5S-18S rRNA complexes could be dis-

sociated by heat. The profile for the temperature dependent dissociation of the barley 5S-18S rRNA complex was very sharp with a "Tm" of ca. 59° in 0.1 M NaCl (Fig. 3a), which is very close to that of the wheat embryo 5S-18S rRNA complex [2]. The mouse sarcoma 5S-18S rRNA complex had a broader transition breadth and a "Tm" of ca. 52° in 0.1 M NaCl (Fig. 3b), suggesting that the mouse sarcoma 5S-18S rRNA complex is thermodynamically less stable and probably less specific than barley and wheat embryo 5S-18S rRNA complexes. Even though the efficiency of in vitro complexing between mouse sarcoma 5.8S and 28S rRNA was low, the melting profiles of the in vivo and in vitro complexes were equally sharp with a "Tm" of ca. 52° (Fig. 3c), which is identical to barley (data not shown) and wheat embryo [2] 5.8S-26S rRNA complexes. Both the barley and mouse sarcoma 5S-18S rRNA and 5.8S-26S (28S) rRNA complexes could be aqueous denatured [8] at room temperature, but could not be dissociated when heated in 0.3 M NaCl at 60°, suggesting that the temperature-dependent dissociation is critically dependent upon ionic strength, as would be expected for base-paired intermolecular complexes [9].

The above results show that reversible base-paired complexes are formed in vitro between 5S and 18S rRNA from barley embryos and mouse sarcoma. Even though the mouse sarcoma 5S-18S rRNA complex is formed less efficiently, and is less stable and probably less specific than the corresponding hybrids from barley and wheat embryo, it is selective. The formation of selective 5S-18S rRNA hybrids in barley embryo, mouse sarcoma, and wheat embryo, and the fact that wheat embryo 5S rRNA hybridizes efficiently with 18S rRNA from yeast, HeLa cells and L cells [2] would suggest that hybridization between 5S and 18S rRNA might be a fairly common phenomenon in eukaryotes, and might have biological significance. As has been stated earlier [1], specific base-pairing between 5S and 18S rRNA, which are present in the 60S and 40S ribosomal subunits, respectively, might at least in part be responsible for the reversible association of ribosomal subunits. Support for this idea comes from the observation that undegraded 5S rRNA is essential for the attachment of the

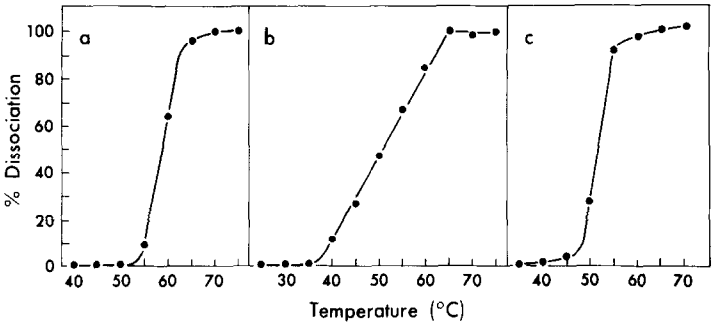

Fig. 3. The temperature-dependent dissociation of the barley embryo [^{3}H] 5S-18S rRNA complex (Fig. 3a), the mouse sarcoma [^{3}H] 5S-18S rRNA complex (Fig. 3b), and the mouse sarcoma [^{3}H] 5.8S-28S rRNA complex, in 0.1 M NaCl. The melting profiles were obtained as described in Methods.

large ribosomal subunit to the small subunit-mRNA-aatRNA complex in Euglena gracilis [10]. Specific ribosomal and/or factor proteins would be expected to play an important role in the formation and stability of 5S-18S rRNA hybrids. The relatively strong interaction between 5S and 18S rRNA from barley and wheat embryo [2] might explain why very low concentrations of Mg^{++} are required to dissociate cereal embryo 80S ribosomes [11,12].

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