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Differences in ribosomal binding activity for polyuridylic acid and f2 bacteriophage RNA between native and derived ribosomal subunits in *Dictyostelium discoideum*

By

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In *Dictyostelium discoideum*, isolated native 40–S ribosomal subunits bound *in vitro* to both polyuridylic acid (as artificial messenger RNA) and f2 phage RNA (as natural messenger RNA), whereas the 40–S subunits derived from 80–S monosomes by lowering the concentration of Mg^{2+} associated with only the former but could not with the latter. No interaction of native or derived 60–S subunits with poly (U) and f2 RNA occurred. It was inferred that the ribosomal particles participating in the formation of the initial mRNA-ribosome complex in eukaryotic protein synthesis are native 40–S subunits.

It is well known that in eukaryotes [1–4] as well as in prokaryotes [5–7] messenger RNA (mRNA) attaches to small ribosomal subunits in polypeptide chain initiation, and that the formation of mRNA-ribosome complex is dependent on the initiation factor(s) which is extracted from ribosomes with high concentrations of NH_4Cl or KCl [1, 2, 5, 7–12]. In *Escherichia coli* all of the initiation factors are present in native 30–S ribosomal subunits but not in native 50–S subunits or 70–S ribosomes, or in the subunits derived from the latter by lowering the Mg^{2+} concentration [13, 14]. These observations provided evidence that the small subunits involved in the formation of mRNA-ribosome complex in bacterial polypeptide chain initiacion are of native type [13–15]. Similar notion is also generally accepted for the case of eukaryotic protein synthesis [4].

In this paper we will report that native and derived 40–S ribosomal subunits of the cellular slime mold *D. discoideum* differ from each other with respect to their binding capacity for polyuridylic acid (poly (U)) and f2 phage RNA.

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Materials and Methods

Amoeba cells of *D. discoideum*, strain NC-4, were grown at 23°C on the standard nutrient medium in association with *E. coli* cells [16]. They were harvested at the late log-phase of growth, washed free from bacterial cells by centrifugation with 14 mM phosphate buffer (pH 6.2) and finally twice with cold Tris-K^+–Mg^{2+} buffer (10 mM Tris-HCl, pH 7.6, 10 mM KCl and 3 mM magnesium acetate), and stored at −30°C until the preparation of cell extract.

80-S ribosomes, native 60-S and 40-S subunits were isolated, as described previously [17, 18], by sucrose gradient centrifugation of the crude cell extract which had been prepared by lysing frozen cells in Tris-K^+–Mg^{2+} buffer containing 6 mM 2-mercaptoethanol followed by centrifugation at 15,000 × *g* for 20 min. The 60-S and 40-S subunits derived from 80-S monosomes by lowering the Mg^{2+} concentration, whech are referred to as "derived" subunits, were prepared by sucrose gradient centrifugation after dialysis of monosomes against 10 mM Tris-HCl (pH 7.6) and 0.1 mM Mg^{2+}-acetate for 10 h at 4°C, as described previously [18].

The growth and purification of f2 bacteriophage were carried out according to the method described by LOEB and ZINDER [19], except that the final purification step by CsCl gradient centrifugation was omitted. Labelling of f2 phage with [^{14}C] uracil and the extraction of f2 RNA were done as described by TAKANAMI *et al.* [20]. Almost all of f2 RNA thus obtained sedimented as a sharp peak at approximately 27 S on a sucrose gradient in 20 mM Tris-HCl (pH 7.6) and 0.14 M NaCl.

In the assay for the ribosomal binding to poly (U) or f2 RNA, [^{3}H] poly (U) or [^{14}C] uracil-labelled f2 RNA were added into the reaction mixture containing ribosomes in 10 mM Tris-HCl (pH 7.8), 10 mM KCl, various concentrations of Mg^{2+}-acetate and 6 mM 2-mercaptoethanol, unless otherwise stated. The mixture was incubated for 10 min at 0°C and then centrifuged at 23,500 rev./min for an appropriate time at 4°C on 10–25% (w/v) sucrose gradients in an SW 25.1 rotor of Spinco L ultracentrifuge. After centrifugation, the gradients were fractionated and the absorbance at 260 nm was determined. The radioactivity was measured in a Beckman liquid scintillation spectrometer as described previously [21].

[^{3}H] poly (U) (8.6 mCi/mmol of uridine) and [^{14}C] uracil (54.8 mCi/mmol) were obtained from Schwartz BioResearch Inc. (U. S.).

Results and Discussion

Native and derived ribosomal subunits were assayed for their binding activity to poly (U) as artificial mRNA. Fig. 1 shows the sucrose gradient centrifugation pattern of the reaction mixture containing native or derived subunits and [^{3}H] poly (U). ^{3}H-count peaks were observed in the regions of native and derived 40–S subunits besides in the region of poly (U) (Figs. 1a & 1c), whereas no distinct radioactive peaks were detected in the native and derived 60–S subunit regions (Figs. 1 a & 1 b). This indicates that both native and derived 40–S subunits interact with poly (U) but neither of two types of the 60–S subunits do. The results are analogous to that by TAKANAMI and OKAMOTO [22] with *E. coli* ribosomal subunits but somewhat at varience with that reported by ROBERTS and COLEMAN [3] who observed in Ehrlich ascites that isolated 60–S subunits bind to poly (U), though they were seven times less effective than isolated 40–S subunits in term of specific binding activity. The quantity of [^{3}H] poly (U) bound per unit amount of native 40–S subunits was increased with a rise of the Mg^+ concentration in the range from 0.5 to 10 mM (data not shown). The effect of the Mg^{2+} concentration on the binding activity of ribosomes could not be tested with derived 40–S subunits, because at more than 1.5 mM Mg^{2+} they were easily associated to form dimers or larger aggregates.

Fig. 1 also shows that at 1.5 mM Mg^{2+} the specific binding activity of native 40–S subunits is about three times greater than that of derived 40–S subunits in respect to the binding activity for poly (U).

Since f2 phage RNA which has been widely used as natural mRNA in *in vitro* experiments of protein synthesis provides two specific loci for ribosomal binding [23], in contrast to synthetic polyribonucleotides interacting with ribosomes randomly [22], attempts were made to assay the ribosomal binding activity to the phage RNA. When native 60–S and 40–S subunits were incubated together with [^{14}C] f2 RNA at 1.5 mM Mg^{2+}, followed by sucrose gradient centrifugation, ^{14}C-count peaks were observed in the 40–S region in addition to the 27–S region in which authentic f2 RNA sedimented (Fig. 2 a). In the assay for [^{14}C] f2 RNA binding of derived 60–S and 40–S subunits, no radioactive peaks were seen in the region other than 27–S (Figs. 2 b & 2 c). The results clearly indicate that f2 RNA binds to native 40–S subunits but not to derived 40–S subunits nor to two types of 60–S subunits either. The f2 RNA-binding activity of native 40–S subunits was not affected by the Mg^{2+} concentration (0.5 mM to 10 mM), in contrast to the case of poly (U) binding. Thus, it

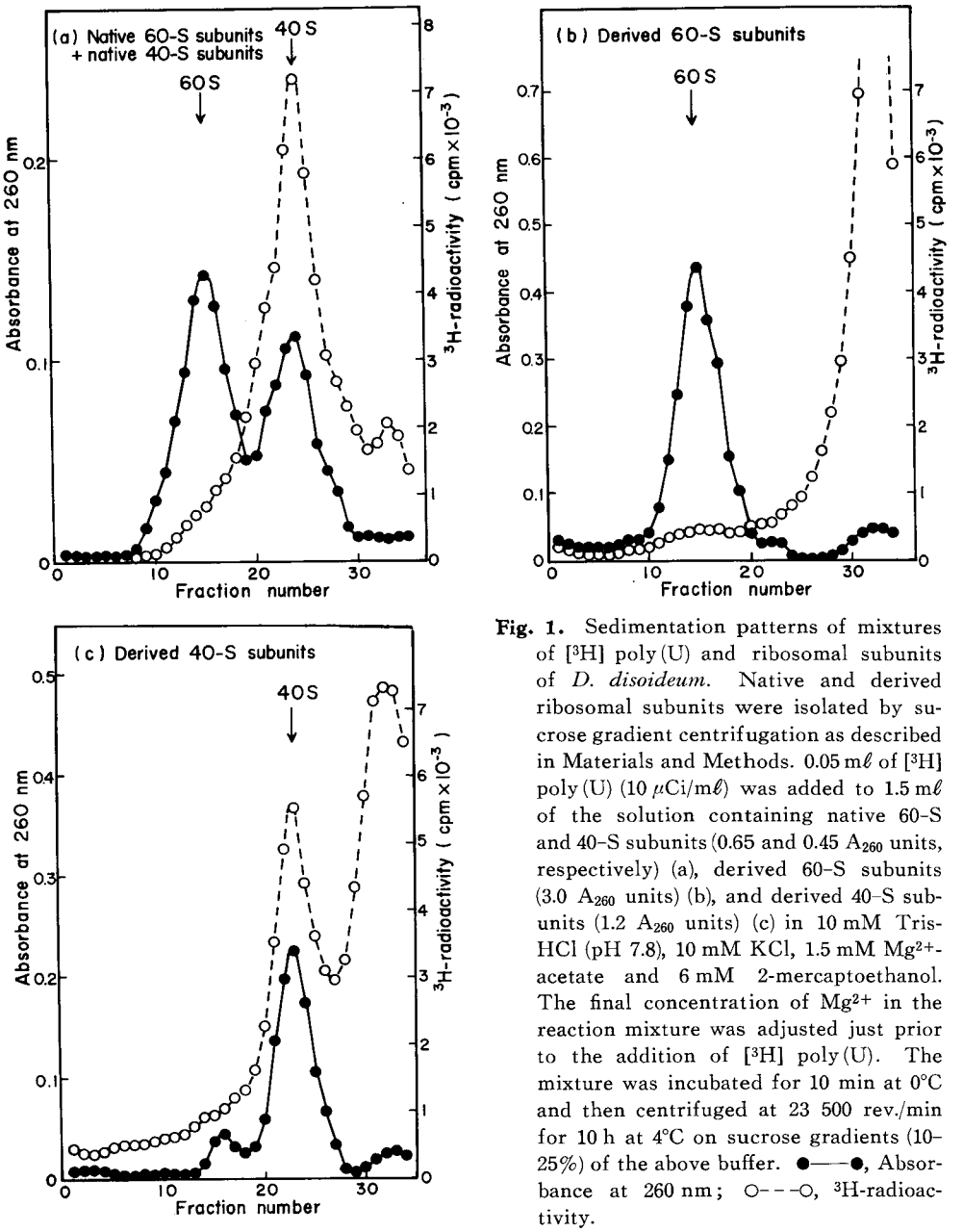

Fig. 1. Sedimentation patterns of mixtures of [^{3}H] poly(U) and ribosomal subunits of *D. disoideum*. Native and derived ribosomal subunits were isolated by sucrose gradient centrifugation as described in Materials and Methods. 0.05 mℓ of [^{3}H] poly(U) (10 μCi/mℓ) was added to 1.5 mℓ of the solution containing native 60-S and 40-S subunits (0.65 and 0.45 A_{260} units, respectively) (a), derived 60-S subunits (3.0 A_{260} units) (b), and derived 40-S subunits (1.2 A_{260} units) (c) in 10 mM Tris-HCl (pH 7.8), 10 mM KCl, 1.5 mM Mg^{2+}-acetate and 6 mM 2-mercaptoethanol. The final concentration of Mg^{2+} in the reaction mixture was adjusted just prior to the addition of [^{3}H] poly(U). The mixture was incubated for 10 min at 0°C and then centrifuged at 23 500 rev./min for 10 h at 4°C on sucrose gradients (10–25%) of the above buffer. ●——●, Absorbance at 260 nm; ○- - -○, ^{3}H-radioactivity.

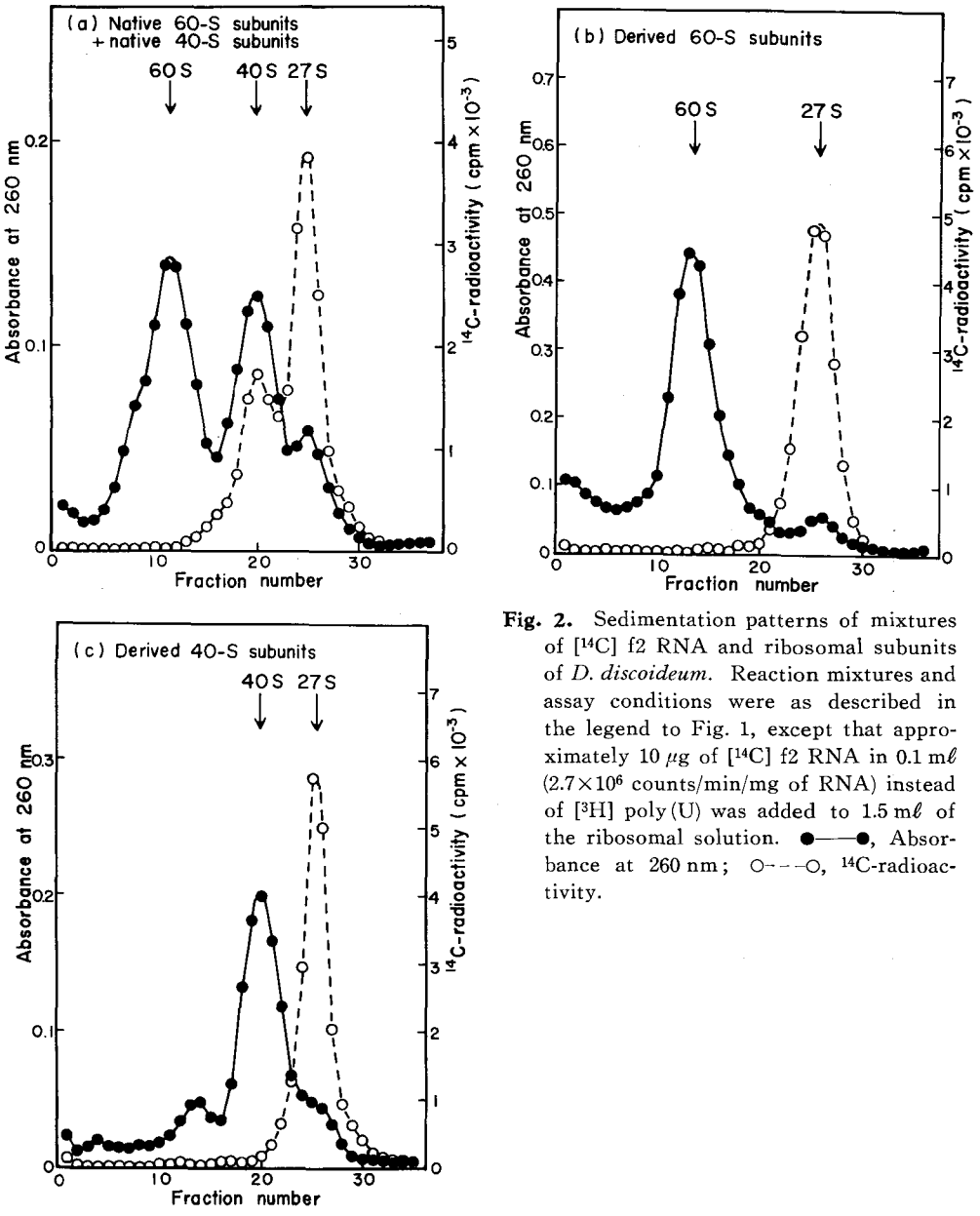

Fig. 2. Sedimentation patterns of mixtures of [^{14}C] f2 RNA and ribosomal subunits of *D. discoideum*. Reaction mixtures and assay conditions were as described in the legend to Fig. 1, except that approximately 10 μg of [^{14}C] f2 RNA in 0.1 mℓ (2.7×10^6 counts/min/mg of RNA) instead of [^{3}H] poly(U) was added to 1.5 mℓ of the ribosomal solution. ●——●, Absorbance at 260 nm; ○- - -○, ^{14}C-radioactivity.

seems certain that there is a difference in the affinity for natural mRNA between isolated native and derived 40–S subunits of *D. discoideum.*

The failure of the 60–S subunits to interact with poly (U) and f2 RNA would be understandable in the light of the recent knowledge about protein synthesis that the binding site of ribosomes for mRNA is located on small subunits [2, 3, 15, 24]. The evidence obtained here certainly supports the notion [1] that the 40–S subunit involved in the formation of the initial mRNA-ribosome complex in eukaryotic protein synthesis is of native form.

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