Short Communications

A Requirement for the Presence of Cell Sap in the Reversible Dissociation of Rat Liver Polysomes to Ribosomal Sub-units

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As part of a study on polysomes in the liver cell we wished to study the conditions required for the interconversion between polysomes and ribosomal sub-units. Whereas bacterial ribosomes may be dissociated into their sub-units after suspension in solutions containing low concentrations of Mg^{2+} ions (Tissières, Watson, Schlessinger & Hollingworth, 1959), it has been reported that decrease of the Mg^{2+} ion concentration, even to as low as $1\mu M$, merely leads to a marked conversion of liver polysomes into monomeric ribosomes (Munro, Jackson & Korner, 1964). Liver ribosomes may be dissociated, however, into sub-units by the addition of EDTA to the suspending medium (Hamilton & Petermann, 1959; Tashiro & Siekevitz, 1965).

The first problem was to study the conditions for the complete dissociation of liver polysomes to ribosomal sub-units and then to determine whether such sub-units could be reconstituted to polysomes. The interconversion was determined by ultracentrifugation and by the ability of 'reconstituted' particles to effect protein synthesis. Since Lawford, Langford & Schachter (1966) have shown that liver cell sap has an important effect on the preservation of polysomes during isolation and Bloemendal, Bont & Benedetti (1964) have shown that cell sap maintains the integrity of polysomes during protein synthesis, our studies included the role of cell sap in the reversible dissociation of liver polysomes.

Polysomes were prepared from the mitochondria-free supernatant of homogenized rat liver by a method similar to that described by Wettstein, Staehelin & Noll (1963) for the preparation of C-ribosomes. The polysomes were carefully suspended in a medium containing $MgSO_4$ (5mM), KCl (25mM) and tris-HCl buffer, pH 7.8 (50mM). The ribosome concentration in the suspension was calculated assuming $E_{1cm}^{1\%}$ 134 at 260m μ . The amount of Mg^{2+} ions in the medium was determined by complexometric titration with a standard solution of EDTA, with Eriochrome Black T as indicator. A solution of EDTA, pH 7.2, was carefully added to the suspension of ribosomes (approx. 2mg./ml.) to exactly compensate for the Mg^{2+} ion content of the medium plus 0.6 μ mole/mg. of ribosomes.

For the determination of the protein-synthetic activity of the ribosome preparations the ribosomes were incubated for 30min. at 37°. The incubation medium contained $MgSO_4$ (13mM), KCl (25mM) and tris-HCl buffer, pH 7.8 (50mM), in the presence of an energy-generating system (see Campbell, Lowe & Serck-Hanssen, 1967), 0.2ml. of cell sap and 0.125 μ C of [^{14}C]phenylalanine (specific radioactivity 495mc/m-mole) in a total volume of 1ml. The incubation was stopped by addition of cold medium and the radioactivity of the protein determined as follows. To a sample was added 4N-NaOH to bring the final concentration to N-NaOH. After 5min. protein was precipitated by the addition of 50% (w/v) trichloroacetic acid. The precipitate was kept cold for at least 1hr. and was then filtered on glass paper (Whatman GFA), rinsed with 5% (w/v) trichloroacetic acid three times and finally washed twice with 3ml. of chloroform-ether-methanol (2:1:1, by vol.). The filter disk with the protein was then placed in a vial containing 10ml. of scintillation fluid 2,5-diphenyloxazole (4g./l. of toluene) and 1,4-bis-(5-phenyloxazol-2-yl)benzene (100mg./l. of toluene). Radioactivity was determined in a Beckman model LS200B liquid-scintillation spectrometer.

The effect of the treatment of liver polysomes under various conditions is shown in Fig. 1. Fig. 1(a) is the sedimentation profile of the polysome preparation that had been suspended in cell sap. Polysomes treated with EDTA in the absence of cell sap and sedimented in a density gradient gave two sharp peaks, which we take to be due to the presence of large and small ribosomal sub-units (Fig. 1b). Polysomes treated with EDTA in the presence of cell sap gave a profile that differed from that obtained in the absence of cell sap in the following respects. The two peaks had a higher rate of sedimentation, had a broader profile and had a lower E_{260}/E_{280} ratio. The gradients contained Mg^{2+} ions at a concentration of 0.1mM, since we have shown that polysomes are not degraded to ribosomal sub-units under these conditions.

When Mg^{2+} ions were re-added to the suspension of EDTA-treated ribosomes to bring the final Mg^{2+} ion concentration to 5mM, the ribosomes had the

Fig. 1. Sucrose-density-gradient analysis of rat liver polysomes after various treatments. The extinction at $260\text{ m}\mu$ was measured in a Gilford model 2000 recording spectrophotometer by pumping the gradients through a flow cell at a constant flow rate of 1 ml./min. (a) A 27 ml. gradient, $15\text{--}50\%$ (w/v) sucrose in tris-HCl buffer, pH 7.8 (50 mm), KCl (25 mm) and MgSO_4 (5 mm), centrifuged at 25000 rev./min. for 4 hr. in a Beckman Spinco SW25.1. The sample was a suspension of untreated polysomes in cell sap. (b) A 20 ml. gradient, $15\text{--}35\%$ (w/v) sucrose in tris-HCl buffer, pH 7.8 (50 mm), KCl (25 mm) and MgSO_4 (0.1 mm), centrifuged at 28000 rev./min. for 13 hr. in an MSE 3×20 rotor., Suspension of polysomes in tris-HCl buffer, pH 7.8 (50 mm), KCl (25 mm) and MgSO_4 (5 mm) treated with EDTA (as described in the text); —, polysomes suspended in cell sap and treated with EDTA. (c) Gradients were the same as in (a). The samples were the same as in (b) except that Mg^{2+} ions were re-added to a final concentration of 5 mm. The arrows mark the position of monomeric ribosomes.

sedimentation profile shown in Fig. 1(c). If cell sap was absent during the addition of EDTA no recombination of ribosomal sub-units took place after the re-addition of Mg^{2+} ions, whereas in the presence of cell sap there was a marked re-formation of ribosomes and most of these were in the form of polysomes. The results obtained when the protein-

synthesizing activity of the preparations was tested supported the conclusions from the sedimentation profiles. Thus incubation of the same amount of ribosomes under the same conditions with $[^{14}\text{C}]$ -phenylalanine gave: untreated polysomes, 5440 counts; 'recombined sub-units' after EDTA treatment in the absence of cell sap, 250 counts; 'recombined sub-units' after EDTA treatment in the presence of cell sap, 5020 counts (all counts are counts/min./mg. of ribosomes after subtraction of zero-time control counts). When polyuridylic acid was added to the incubation medium the effect was similar in all cases, the ratios being 1.1, 1.1 and 1.3 respectively.

Dissociated bacterial ribosomes may be re-constituted in the presence of Mg^{2+} ions and the ribosomes produced are active for protein synthesis (e.g. Staehelin & Meselson, 1966). However, reconstitution of bacterial polysomes does not appear to have been demonstrated. Tashiro & Siekevitz (1966) failed to reconstitute sub-units of guinea-pig liver ribosomes under similar conditions. When rabbit reticulocytes are incubated in a medium containing NaF the polysomes dissociate to monomers (Marks, Burka, Conconi, Perl & Rifkind, 1965; Conconi, Bank & Marks, 1966). Pronczuk, Baliga, Triant & Munro (1968) have reported that rat liver polysomes may be reversibly converted into monomers *in vitro* and that the composition of the amino acid mixture in the incubation medium used for amino acid incorporation into protein has a marked effect on such a process.

The present experiments show that polysomes can be re-formed from ribosomal sub-units provided that cell sap is present during the dissociation of ribosomes in the presence of EDTA (it is not sufficient to add the cell sap after completion of the addition of EDTA). The nature of the ribosomal particles present after the addition of EDTA in the presence of cell sap is not precisely known, but clearly they are ribosomal sub-units. These seem to be associated with more protein than is the case when cell sap is absent and, since ribosomes can only be obtained from the sub-units that have been prepared in the presence of cell sap, we think that the 'extra' protein must be important. The re-formation of polysomes would be expected to require messenger RNA. Experiments with pulse-labelled RNA support the view that the messenger RNA remains with the particles after treatment with EDTA in the presence of cell sap, as also shown by Nolan & Arnstein (1968) for reticulocyte ribosomes. That true polysomes linked with messenger RNA, rather than ribosomal aggregates, are re-formed is indicated by the lack of an enhanced effect of polyuridylic acid with the 'recombined' polysomes compared with the untreated particles. The exact sequence of events in the interconversion

of polysomes and ribosomal sub-units is not yet clear, e.g. whether monomers are an essential intermediate. However, the results of others (Pronczuk *et al.* 1968), showing that ribosomes may be converted into polysomes, suggest that monomeric ribosomes are an intermediate.

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