The Phosphorylation of an Acidic Protein of the Large Ribosomal Subunit of Krebs II Ascites Cells

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The ribosomes of Krebs II ascites cells contain an acidic protein, apparently analogous to proteins L7/12 of *Escherichia coli*. When ascites cells were incubated with [32 P]P_i, this protein became labelled, indicating that it is a phosphoprotein.

The proteins designated L7 and L12 are among the most interesting and well characterized of the ribosomal proteins of *Escherichia coli*. Ribosomal protein L7 is, in fact, the *N*-acetylated derivative of L12, and these two unusually acidic ribosomal proteins are unique in together being present in three or four copies per large ribosomal subunit. Moreover, there is strong evidence that these proteins interact with some of the soluble factors which catalyse the partial reactions of protein synthesis [see Möller (1974) for a review]. The functional importance of proteins L7/12 is further indicated by the finding that these are the only ribosomal proteins of *E. coli* which have immunologically cross-reacting counterparts in eukaryotes (Stöffler *et al.*, 1974). We have been studying the phosphorylation of eukaryotic ribosomal proteins and report here that when resting Krebs II ascites cells are incubated with [32 P]P_i, the protein (or proteins) apparently analogous to L7/12 of *E. coli* becomes phosphorylated.

Experimental

Krebs II ascites cells, passaged in Porton-strain mice, were suspended at a concentration of 1×10^7 – 2×10^7 cells/ml of medium [which was Earle's saline (Earle, 1943) supplemented with 10% (v/v) calf serum (Gibco Bio-Cult, Glasgow, Scotland, U.K.)] and incubated with [32 P]P_i (0.1 mCi/ml) for 3 h at 37°C. Ribosomes were then isolated, their subunits prepared and the protein was extracted, all as previously described (Rankine & Leader, 1975).

Our miniaturization of the two-dimensional gel-electrophoretic system of Kaltschmidt & Wittmann (1970), using a pH of 8.6 in the first dimension, has also been described (Leader, 1975). In the experiments presented here the pH of the first-dimension gels was lowered to pH 6.6 by decreasing the proportion of EDTA, and 0.01 M-bis-(2-hydroxyethyl)imino-tris-(hydroxymethyl)methane (Bis-Tris), adjusted to pH 6.6 with acetic acid, was used as running buffer. Proteins were identified by their electrophoretic

mobilities according to the nomenclature of Sherton & Wool (1974). In some experiments a second dimension containing 0.1% sodium dodecyl sulphate, 6M-urea and 10% acrylamide was used (Martini & Gould, 1971), and gels were run in parallel with proteins of known molecular weight (bovine serum albumin, chymotrypsinogen and cytochrome *c* of mol.wts. 64000, 27500 and 12500 respectively).

Results and Discussion

Although it has been clearly established that the acidic proteins of rat liver designated L40/41 by Sherton & Wool (1974) are the eukaryotic equivalents of proteins L7/12 of *E. coli* (Stöffler *et al.*, 1974), several workers have been unable to detect these in other eukaryotic ribosomes (Creusot *et al.*, 1975; Howard *et al.*, 1975; Terao & Ogata, 1975). Indeed, in previous studies we had also been unable to find such ribosomal proteins in either rat liver (Leader, 1975) or ascites cells (A. D. Rankine & D. P. Leader, unpublished work) by using the standard two-dimensional gel-electrophoretic system of Kaltschmidt & Wittmann (1970), with pH 8.6 in the first dimension. However, in studies where the proteins of the large ribosomal subunit of ascites cells were separated in gels in which the pH of the first dimension was lowered to 6.6, a weakly staining acidic protein corresponding to L40/41 was clearly visible (Plate 1). Although our detection of only one acidic protein in ascites cells contrasted with the finding of two in rat liver (Sherton & Wool, 1974), it was similar to the results for skeletal-muscle ribosomes in this latter study. Because the acidic ribosomal protein of ascites cells has a similar electrophoretic mobility to proteins L40/41 of rat liver, we have designated it as protein L40/41. Although we are fairly confident that the proteins are analogous, we appreciate that immunochemical studies are needed to confirm this.

We also analysed the acidic protein of ascites cells on polyacrylamide gels in which the second dimen-

sion contained sodium dodecyl sulphate to allow estimation of the molecular weight of the protein (Plate 2a). The value of 13500 obtained was lower than that of 25500 obtained by Lin & Wool (1974) for proteins L40/41 from rat liver, but this discrepancy is probably more apparent than real, being consistent with a higher range of values for the molecular weights of eukaryotic ribosomal proteins obtained by these workers when compared with the values found by ourselves and others (e.g. Howard *et al.*, 1975). Our, lower, value agrees well with that of 13000 for the analogous acidic proteins of *Artemia salina* (Möller *et al.*, 1975), and these are similar to that of 12000 for proteins L7/12 from *E. coli* (Terhorst *et al.*, 1973). If correct, this suggests that the functional similarity between proteins L40/41 and L7/12 has resulted in a conservation of size during evolution, in apparent contrast with other eukaryotic ribosomal proteins.

Plate 2(b) shows that proteins L40/41 are phosphorylated when intact ascites cells are incubated with [32 P]P_i. The phosphorylation of these functionally important proteins is of obvious interest in view of the well-characterized examples of changes in the biological activity of other proteins after phosphorylation [see Rubin & Rosen (1975) for a review]. However, it should be emphasized that only about 10% of the 0.36 mol of phosphate/mol of 60S subunit is contained in proteins L40/41, the majority being associated with the protein designated L₇ in Plate 1 (Rankine *et al.*, 1977). Although this might mean that there is only an unimportant trace phosphorylation of proteins L40/41, a functional role for the phosphorylation cannot be excluded. Thus one possibility is that phosphorylation and dephosphorylation of proteins L40/41 might occur during each round of protein synthesis, but only a small proportion of the molecules of proteins L40/41 are isolated in the phosphorylated state. An alternative possibility is that proteins L40/41 are, in fact, quite extensively phosphorylated, but that the amount of L40/41 detected on the two-dimensional gels represents only a small proportion of the total. This might be due to loss of proteins L40/41 from ribosomes during preparation (Stöffler *et al.*, 1974) or to their aggregation with another ribosomal protein or proteins, as reported for proteins L7/12 in *E. coli* (Pettersson *et al.*, 1976). In this latter regard, it should be noted that the mol.wt. of 13500 reported here for proteins L40/41 is similar to that previously found by us for the major phosphoproteins of the 60S subunit, a closely migrating doublet (Leader & Rankine, 1976; Rankine & Leader, 1976). It is thus conceivable that the protein L₇ (Plate 1), which contains most of the radioactive phosphate, is an aggregate containing phosphorylated proteins L40/41, and immunochemical experiments are needed to test this possibility.

Finally it is worth remarking that, although proteins L7/12, in common with other prokaryotic ribosomal proteins, are not phosphorylated *in vivo* (Gordon, 1971), they may be phosphorylated by using a eukaryotic protein kinase *in vitro* (Issinger & Traut, 1974). Moreover, since completing this work, we have learned that Zinker & Warner (1976) have detected the phosphorylation of an acidic ribosomal protein in *Saccharomyces cerevisiae*.

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EXPLANATION OF PLATE I

An acidic protein in the large ribosomal subunit of ascites cells

Protein (200 μ g) from the 60S subunit of the ribosomes of Krebs II ascites cells was subjected to standard two-dimensional gel electrophoresis, with a modified first dimension at pH 6.6, as described in the Experimental section. The illustration shows parallel gels of the acidic (a) and basic (b) proteins, stained with Coomassie Brilliant Blue.

EXPLANATION OF PLATE 2

Phosphorylation of the acidic ribosomal protein of ascites cells

Protein (200 μ g) from the 60S subunit of the ribosomes of Krebs II ascites cells incubated with [³²P]P_i was subjected to two-dimensional gel electrophoresis in which the acidic protein was run at pH 6.6 in the first dimension and in a second dimension containing sodium dodecyl sulphate, as described in the Experimental section. The scale of molecular weights was obtained from parallel second-dimensional gels of proteins of known molecular weights. (a) Gel stained with Coomassie Brilliant Blue; (b) radioautograph [obtained as described by Rankine & Leader (1975)].