ACIDIC PHOSPHOPROTEINS OF HELA AND RAT 60 S RIBOSOMAL SUBUNITS

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1. Introduction

It has been argued that the acidic phosphoproteins of 60 S ribosomal subunits from *Saccharomyces cerevisiae* [1] and from HeLa cells [2,3] may be equivalent to the proteins L40/L41 of rat liver ribosomes [4,5] and EL7/EL12 of *Artemia salina* ribosomes [6]. Their homology to the proteins L7/L12 of *Escherichia coli* (*E.coli*) ribosomes has been suggested [4,7]. Additional protein spots, A1–A4, in the anodic part of the gels from 60 S subunits have recently been described [8]. It has been proposed [5] that these spots, rather than L40/L41, are the equivalents of the *E. coli* proteins L7/L12. The spots L40/L41 [4], recently referred to as AX2/AX3 [8], may have arisen as aggregates of the proteins A1–A4 [5]. Such aggregation could account for the observed differences in the molecular weights of the rat liver proteins L40/L41 (mol. wt. 25 500) [9] and the apparent L7/L12 homologous proteins (mol. wt. 12 000–14 000) from other eukaryotic cells [1,3,6].

We have reinvestigated the apparent differences between rat liver 60 S acidic proteins and those of other eukaryotes by taking advantage of the in vitro phosphorylation of HeLa L40 proteins [3].

2. Experimental

Ribosomes and ribosomal subunits were prepared from HeLa cells as described previously [2,3] and from rat liver as described by Sherton and Wool [10]. The Kaltschmidt-Wittmann two dimensional polyacrylamide gel electrophoresis and electrophoresis in dodecylsulfate-containing gels were performed as described [11,3]. In vitro phosphorylation of ribosomal proteins with HeLa protein kinase and [γ-^{32}P]GTP has previously been published [3]. Poly(U) directed polyphenylalanine synthesis was carried out according to Reyes et al. [5].

3. Results and discussion

HeLa protein kinase phosphorylates the proteins L40/L41 in vitro when 60 S subunits from HeLa cells are used as a substrate [3]. The same pattern of phosphorylation is seen when rat liver 60 S subunits are incubated with HeLa protein kinase and [γ-^{32}P]GTP (fig.1) or [γ-^{32}P]ATP. The observation that the same proteins of HeLa and rat 60 S subunits are phosphorylated in vitro can be used to clarify the possible interrelationship among the rat liver acidic proteins. If the AX2/AX3 proteins (mol. wt. 25 500) represent aggregates of two molecules of the A1–A4 proteins (mol. wt 13 700), which may be generated during the extraction procedure, they should be labelled and should be separable by two dimensional polyacrylamide gel electrophoresis. (In fig.1, whole ribosomes were used without protein extraction.) Figure 2 shows that the two major spots AX2/AX3 of rat liver 60 S subunits are not phosphorylated in vitro and therefore could not originate from the phosphoproteins A1–A3 by aggregation. The possibility of aggregation of only the unphosphorylated form (A4) is unlikely but not excluded. The identity of labelled proteins from fig.2 with the phosphoproteins in fig.1 has been demonstrated by re-running the protein spots cut out from two dimensional gels in dodecylsulfate containing gels (data not shown). The comparison of the stained gel from fig.2a with the stained gel of HeLa 60 S subunits (fig.3) shows that there is no protein spot on HeLa ribosomes corresponding to the rat proteins AX2/AX3.

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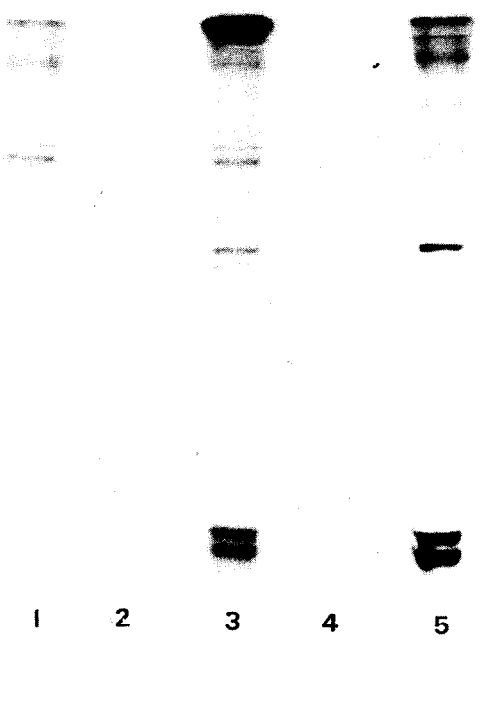

Fig.1. In vitro phosphorylation of 60 S ribosomal subunits from rat liver and HeLa cells. 0.5 M KCl-washed 60 S subunits were incubated in the reaction mixture with HeLa protein kinase and [γ-^{32}P]GTP and analyzed on polyacrylamide gel slabs as described [3]. An autoradiograph of the dried gel is shown. Track 1, enzyme without ribosomes; track 2 and 3, 60 S rat subunits; track 4 and 5, 60 S HeLa subunits. Track 2 and 4 without enzyme; track 3 and 5, with enzyme.

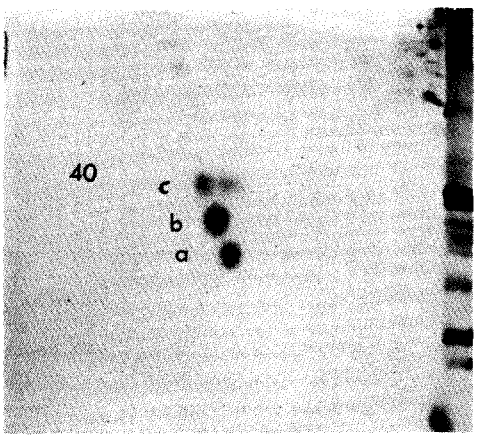

Fig.3.Acidic proteins of the 60 S HeLa ribosomes. 0.5 M KCl-washed HeLa 60 S subunits were analyzed by two dimensional electrophoresis [3] and stained with Coomassie Blue.

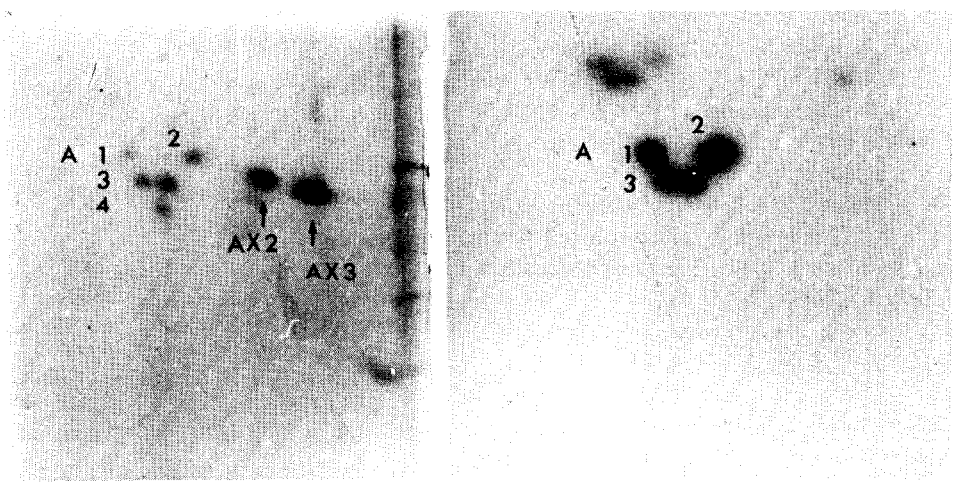

Fig.2. Two dimensional analysis of acidic phosphoproteins from in vitro labelled rat 60 S subunits. 60 S ribosomal subunits were phosphorylated in vitro as described in the legend to fig.1. Ribosomal proteins were extracted and separated as described [3]. (A) Coomassie Blue stained gel; (B) autoradiograph.

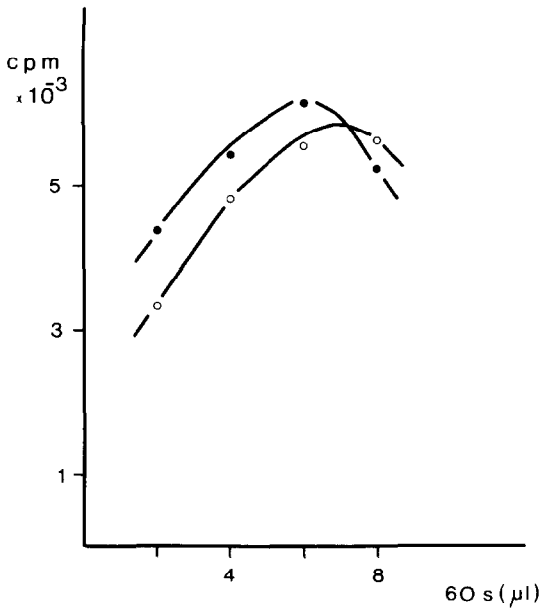

Fig.4. Activities of HeLa and rat 60 S subunits in poly (U)-directed polyphenylalanine synthesis. 40 S rat subunits were incubated with increasing amounts of rat (•——•) and HeLa (○——○) 60 S subunits in the reaction mixture as described [5].

The proteins from rat liver ribosomes named A1–A4 [8] here and named L40/L41 by other authors [5] and the proteins L40/L41 from HeLa cells [3] are the main components of the 'split protein fraction' which can be washed off the ribosomes by treatment with 1 M NH_4Cl–50% ethanol [4,5]. It has been previously shown that rat liver 80 S ribosomes deprived of the 'split protein fraction' are inactive in polyphenylalanine synthesis [5]. Furthermore, antisera against *E.coli* proteins L7/L12 inhibit polyphenylalanine synthesis in chicken liver systems [7] indicating that the eukaryotic equivalents of the *E. coli* proteins L7/L12 are indispensable for ribosomal function [4,7].

We have therefore asked whether or not AX2/AX3 proteins from HeLa ribosomes are lost during their isolation. If washed HeLa 60 S subunits do not possess these proteins they should not be active in the in vitro phenylalanine-polymerizing system. In the experiment described in fig.4, we have compared rat and HeLa 60 S subunits in the polymerization of phenylalanine, and have found them equally active. This observation indicates (1) that HeLa 60 S subunits must possess proteins homologous to *E.coli* proteins L7/L12, and (2) that these proteins are not functionally equivalent to the proteins AX2/AX3 from rat liver ribosomes.

Our results support the notion of Reyes and coworkers [5] that the proteins equivalent to the *E.coli* proteins L7/L12 are the rat liver proteins named A1–A4 here. From the homology of the rat liver proteins A1–A4 with the HeLa proteins L40/L41 and from the fact that rat liver proteins can be phosphorylated also in vitro, we assume that these proteins are also phosphorylated in vivo. Two additional observations support the speculation that the proteins A1–A4 of rat liver or L40 a, b, c of HeLa cells are homologous to *E. coli* proteins L7/L12. First, the molecular weight of these proteins are in good agreement with the molecular weight of the *E.coli* proteins L7/L12. Second, the *E.coli* proteins L7/L12 can also be phosphorylated in vitro [12].

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