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PHOSPHORYLATION OF PROTEINS OF RIBOSOMES AND NUCLEOLAR PRERIBOSOMAL PARTICLES IN VIVO IN NOVIKOFF HEPATOMA ASCITES CELLS*

Archie W. PRESTAYKO, Mark O. J. OLSON and Harris BUSCH
Nuclear Protein Laboratory, Department of Pharmacology, Baylor College of Medicine, Houston, Texas 77025, USA

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1. Introduction

Phosphorylated proteins are found in ribosomes [1, 2] and in nucleoli [3, 4] of a number of cell types. Since ribosomes are assembled and processed in the nucleolus, it was of interest to determine the relationship between the phosphorylation of ribosomal proteins present in the nucleolus [5, 6] and the phosphorylation of proteins of cytoplasmic ribosomes in Novikoff hepatoma ascites cells. It was found that different proteins are phosphorylated in the ribosomes and in the nucleolar preribosomal particles. Furthermore, most of the acid soluble phosphorylated proteins of the preribosomal particles are nonribosomal and are different from the chromatin bound phosphoproteins.

2. Materials and methods

Novikoff hepatoma cells were labeled for 2 hr by intraperitoneal injection of 20 mCi/rat of carrier-free [^{32}P]orthophosphate. Nucleoli were prepared by the sonication procedure [3]. Preribosomal particles and ribosomes were prepared as previously described [5]. To prepare nucleolar chromatin, nuclei were first prepared by the citric acid method [7]. Nucleoli obtained by sonication were washed twice with 10 volumes of 0.025 M EDTA, 0.075 M NaCl, 5 mM $NaHSO_3$, pH 8.0, and 3 times with 10 vol of 0.01 M Tris–HCl, pH 8.0 [8].

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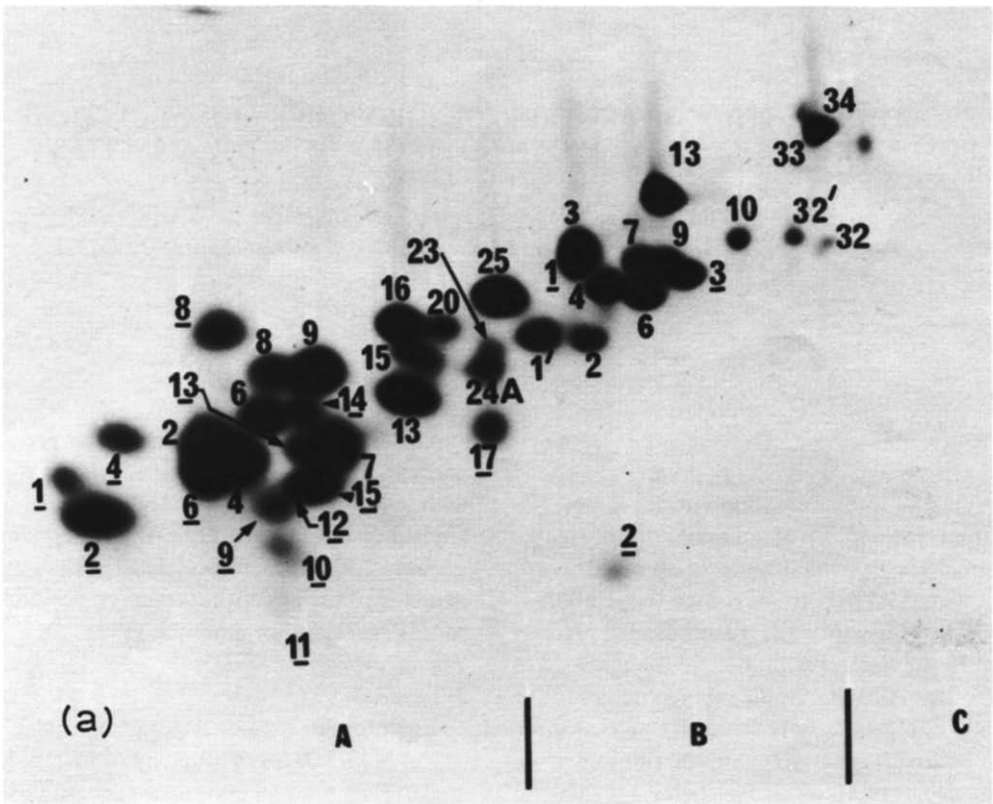
Proteins from ribosomes, nucleolar preribosomal particles, or nucleolar chromatin were extracted twice with 0.4 N H_2SO_4 precipitated with ethanol and applied to the two-dimensional polyacrylamide gel system [3,9]. Radioactively-labeled proteins were detected in the gels by autoradiography and analyzed for ^{32}P-labeled phosphoserine [3].

3. Results

The proteins extracted from ^{32}P-labeled ribosomes by 0.4 N H_2SO_4 were generally of low molecular weight (less than 50 000) and migrated in the A and B regions of the two-dimensional gel system (fig. 1a). By autoradiography (fig. 1b), four spots of radioactivity were found which coincided with ribosomal proteins B2, B3, B6 and B32. A fifth spot, B35P (P indicates radioactive spots that do not co-migrate with stained protein spots), did not coincide with a stained protein spot. All radioactive spots analyzed for phosphoserine contained ^{32}P-labeled phosphoserine which accounted for more than 90% of the radioactivity in most spots.

Fig. 2a indicates that approximately 80 protein spots were obtained from the extract of nucleolar preribosomal particles. After autoradiography of the two-dimensional slab gel, approximately 19 radioactive spots were found (fig. 2b). A few faint spots (A25, A24A, A7 and A2P) were detected only after long exposure times and some spots (A2P, A6-9P, A17P, B12P$_1$, B12P$_2$, B27P, C3P and C9P) did not coincide with stained spots. Four of the proteins (A7, A24A, A25 and B13) were previously found to be present in cytoplasmic ribosomes [5].

In order to determine whether some of the phos-



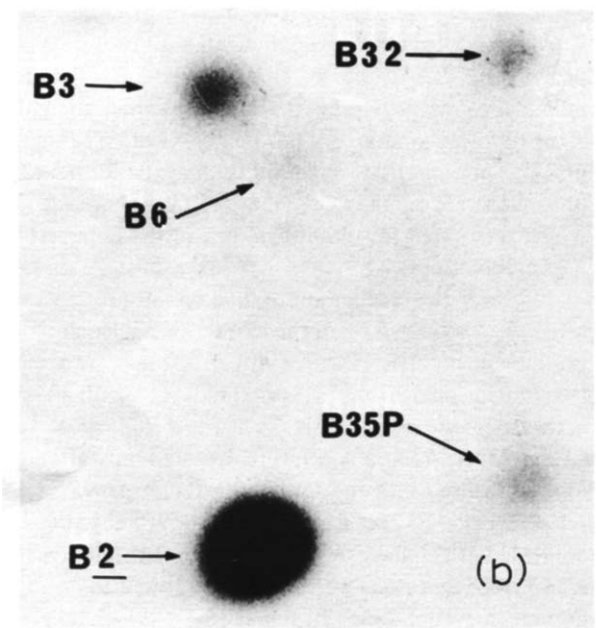


Fig. 1 a: Two-dimensional polyacrylamide gel electrophoresis of 250 μg of Novikoff hepatoma polyribosomal protein run and numbered as previously described [5,9]; b: Autoradiogram on X-ray film of ^{32}P-labeled ribosomal proteins subjected to two-dimensional gel electrophoresis as in fig. 1a. The numbers correspond to the stained spot numbers in fig. 1b. Numbers followed by P indicate radioactive spots that do not co-migrate with stained spots.

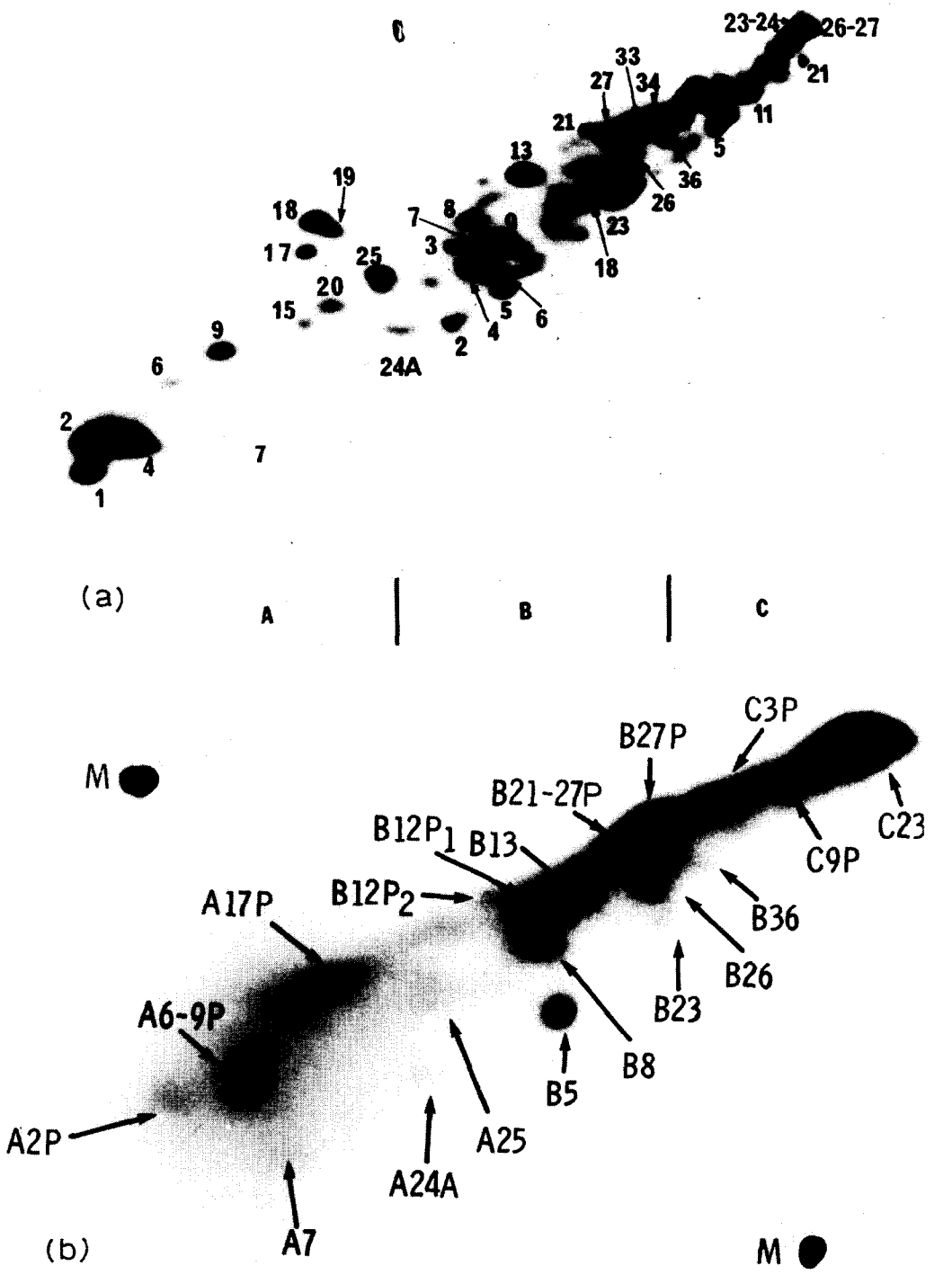


Fig. 2.a: Two-dimensional polyacrylamide gel electrophoresis of 250 μg of proteins from Novikoff hepatoma nucleolar preribosomal (nucleolar RNP) particles; b: Autoradiogram on X-ray film of ^{32}P-labeled nucleolar preribosomal proteins subjected to two-dimensional gel electrophoresis as in fig. 2a. M refers to radioactive marker spots.

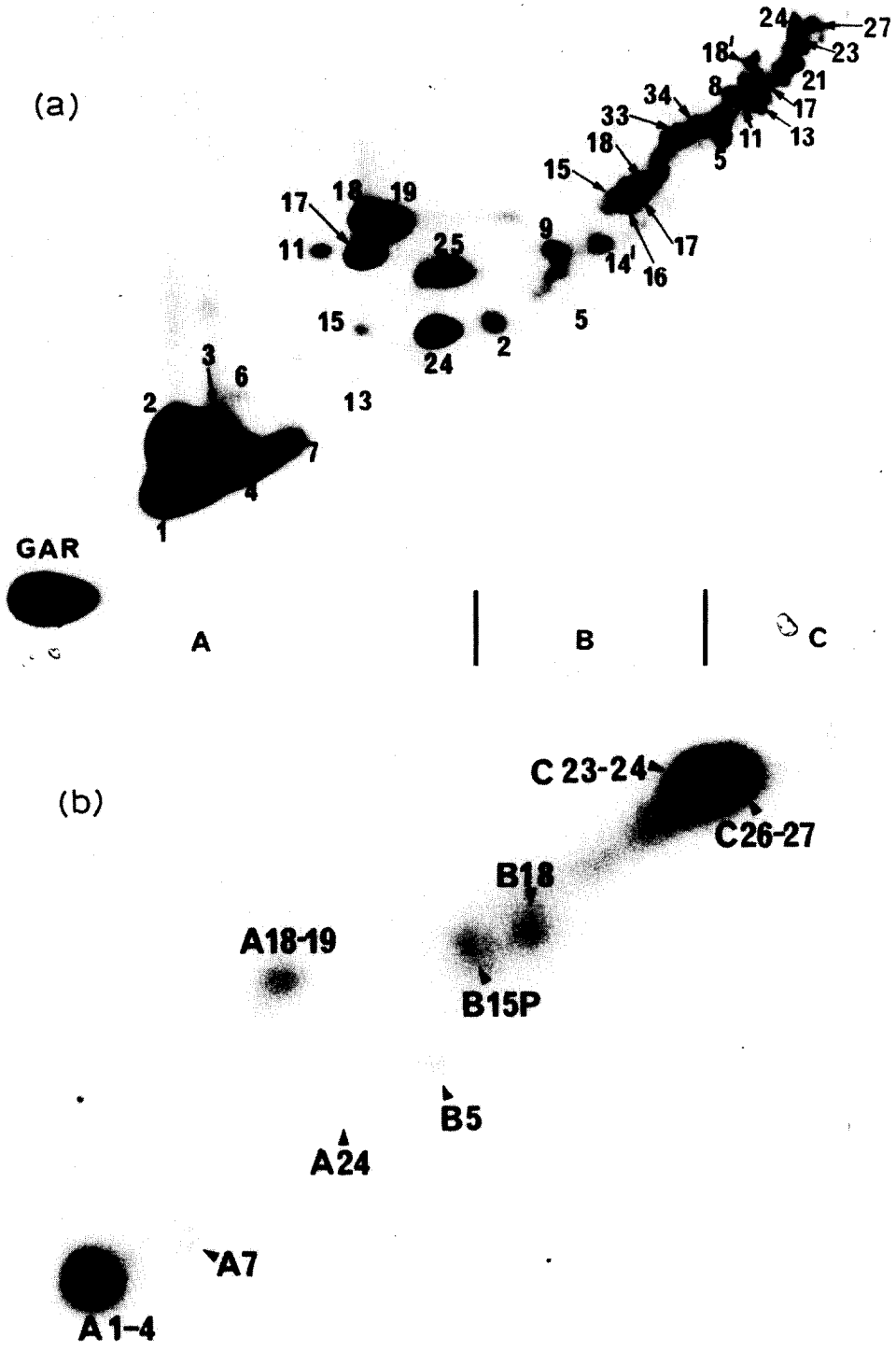


Fig. 3.a: Two-dimensional gel electrophoresis of 0.4 N H_2SO_4 soluble proteins of nucleolar chromatin. b: Autoradiogram of gel slab of fig. 3a.

phorylated proteins of nucleolar preribosomes were present in nucleolar chromatin, acid extracts of nucleolar chromatin were analyzed by two-dimensional gel electrophoresis. Although many of the proteins of nucleolar chromatin had identical electrophoretic mobilities to proteins of preribosomal particles (fig. 3a), only three (C23-24, B5 and A7) were common phosphorylated proteins (fig. 3b). The other radioactive spots were found only in acid extracts of nucleolar chromatin.

4. Discussion

The phosphorylation of proteins was determined for three different cellular fractions, i.e. ribosomes, nucleolar preribosomes and the acid extract of nucleolar chromatin.

In comparing ribosomes and nucleolar preribosomes it was found that no common proteins were phosphorylated. There results suggest that it is unlikely that phosphorylation of ribosomal proteins in the nucleolus is concerned with cytoplasmic functions of ribosomes. Apparently, ribosomal proteins which are phosphorylated in the nucleolus are dephosphorylated before they become part of the cytoplasmic ribosomes. The majority of the proteins phosphorylated in preribosomal particles are non-ribosomal proteins [5] similar in molecular weight to the 'nucleolar stable' proteins described by Kumar and Warner [11] and Soeiro and Basile [12]. These phosphoproteins may be important components of the assembly and processing system for nucleolar preribosomal particles.

Of the proteins phosphorylated in the chromatin fraction, only three spots (C23-24, B5 and A7) are also phosphorylated in preribosomes. Other phosphoproteins of the chromatin acid extract include C27-27, B18, B15P, A7, A24 in addition to Spots A1-4 and A18-9 which were previously shown to comigrate with histones [10]. Although the majority of high molecular weight proteins of nucleolar preribosomes are removed during preparation of chromatin from nucleoli, several proteins with the same electrophoretic mobilities as proteins from preribosomes (C23, C21, C5, C11, B33, B34, B9, B18, B2, A25) remain associated with chromatin.

These results show that in addition to histones, acid extracts of nucleolar chromatin contain a number of proteins, some of which are present in preribosomes. Some of these proteins may bind to newly synthesized ribosomal precursor RNA in the fibrillar elements of nucleoli [13].

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