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RIBOSOMAL PHOSPHOPROTEINS IN PHYSARUM POLYCEPHALUM

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SUMMARY

Ribosomal proteins of Physarum polycephalum were labelled in vivo with $^{32}PO_4$. Three acid phosphoproteins were observed in the large subunit, while two basic ones were present in the small subunit. Ribosomal phosphoprotein S3 accounted for 70% of the total radioactivity and may be equivalent to S6 from rat liver.

INTRODUCTION

The phosphorylation of eukaryotic ribosomal proteins in vivo has been reported in many recent studies (1-6). Changes in the level and number of phosphoproteins occur after viral infection (7), addition of hormones (8), c-AMP (9), partial hepatectomy (2), or during the cell cycle (10). Many results suggest that this phenomenon may be involved in the control of protein synthesis.

Studies of protein synthesis in vitro with normal and phosphatase treated ribosomes have not shown any correlations with aminoacyl-tRNA binding, peptide bond formation, or initiation on a natural template. However, the termination and initiation with a cell specific mRNA has not yet been tested (11).

In vitro phosphorylation of ribosomes with purified protein kinases has been of limited use. Generally, more proteins were labelled than in vivo and it will have to be shown proven if the phosphorylated proteins were modified at the same site in vitro as in vivo (12).

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It seems that further progress could be accomplished by the establishment of more precise correlations between the physiological state of cells and ribosome phosphorylation.

The acellular slime mold Physarum polycephalum can be grown in axenic cultures as large plasmodia containing up to 10^8 nuclei dividing by mitosis with an exact natural synchrony. Various states of differentiation (spherulation, sporulation, germination) can be studied easily in the laboratory (13). This primitive eukaryote can therefore be considered as a model system for the investigation of events occuring during the cell cycle and differentiation.

The ribosomal proteins of this organism have been analyzed recently (14). Results presented in this report show that five ribosomal proteins were labelled in vivo by $^{32}PO_4$. Three phosphoproteins were acidic and present in the large subunit. The small subunit contained the two basic ones. One of these proteins may be related to S6 from rat liver (2). Combined two-dimensional and SDS gel electrophoresis enabled us to ascertain that each radioactive spot effectively contained only one protein.

The results show that Physarum polycephalum will be an interesting organism in which to study ribosomal protein phosphorylation.

MATERIAL AND METHODS

Submerged cultures of Physarum polycephalum, strain M_3C, were grown under continual agitation at 26°C in 500 ml flasks containing 50-200 ml of semi-defined medium (13). Cells were labelled for at least 24 hours by addition of 100 µCi/ml of $^{32}PO_4$ to the media in which the KH_2PO_4 had been omitted (15).

Ribosomes were purified as previously described (14). Briefly, microplasmodia were collected by a gentle centrifugation for 2 minutes at 300 g, after the cell density reached 4-5% v/v. They were then suspended in 2-2.5 volumes of cold buffer TB (0.2M Sucrose; 0.1M KCl; 0.1M $MgCl_2$; 0.025M EDTA; 0.05M Tris-HCl; pH 7.2) and broken with four strokes in a Potter equipped with a Teflon pestle. The

homogenate was centrifuged for 10 minutes at 15,000 g in a Sorvall SS-34 rotor. The post mitochondrial supernatant was transferred into a 50.2 Ti rotor (Beckman) and the ribosomes were pelleted for 3 hours at 49,000 rpm. The supernatant was discarded and the pellets resuspended in half the initial volume of buffer TB. This crude ribosome preparation was contaminated with polysaccharide, which was elimated by a centrifugation of 30 minutes at 39,000 rpm. The supernatant, containing most of the ribosomes, was centrifuged for 2 hours at 49,000 rpm.

The ribosomal proteins were extracted with acetic acid (16) and precipitated with acetone (17). Residual ribosomal RNA was eliminated by incubation of the proteins with a mixture of pancreatic and T_2 ribonucleases (6). Two-dimensional electrophoresis was performed according to Kaltschmidt and Wittmann (18). The phosphoproteins were localized by autoradiography and further analyzed by SDS-electrophoresis (19) on a 12-16% gradient of polyacrylamide gel according to Laemmli (20).

RESULTS AND DISCUSSION

The two-dimensional gel electrophoresis enabled us to resolve the ribosomal proteins into 67 spots. A photograph of the gel is shown in Figure 1A. When $^{32}PO_4$ labelled proteins were used, five spots could be detected by autoradiography (Fig. 1B), namely S3, S9, L1, L20, L24. Five radioactive bands could also be detected after the electrophoresis of the ribosomal proteins in presence of SDS (Fig. 2). In order to confirm that these bands were effectively phosphorylated proteins, a sample of the labelled material was incubated with pronase prior to electrophoresis. Results showed that the bands disappeared from the gel and only diffuse staining could be seen near the front of migration. Autoradiography of the gels gave a similar pattern (Results not shown). It can therefore be concluded that the five bands detected by autoradiography were effectively ribosomal phosphoproteins.

In order to correlate the data obtained by two-dimensional gel electrophoresis and SDS electrophoresis, the individual labelled spots were cut out from the two-dimensional gel and submitted to electrophoresis in SDS gels. The results are summarized in Table 1.

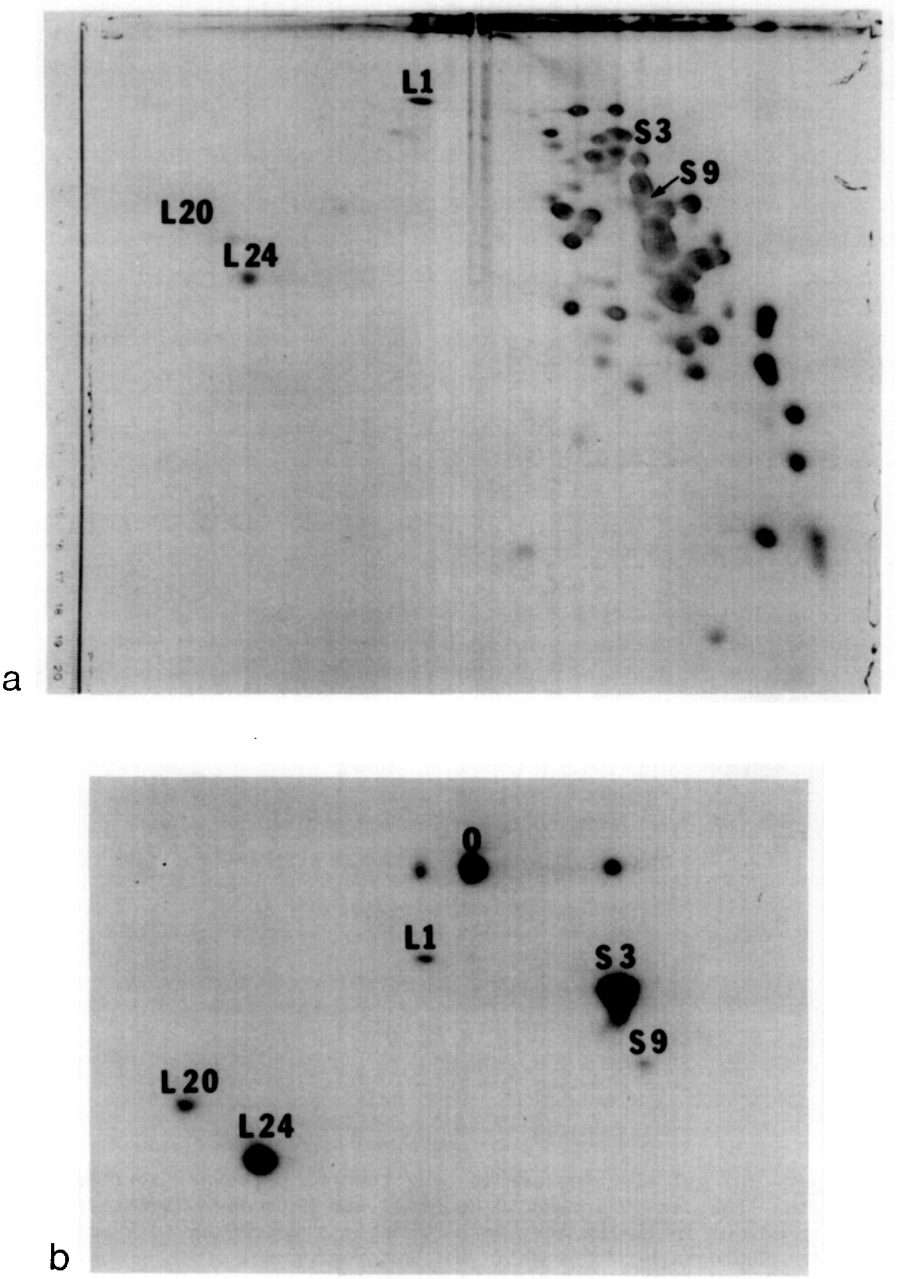

Figure 1. Two dimensional electrophoresis of total ribosomal proteins (1.2 mg) according to Kaltschmidt and Wittmann (18). A: Photograph of the gel after staining. B: Autoradiography of the gel after labelling the proteins in vivo with $^{32}PO_4$ as described in Material and Methods.

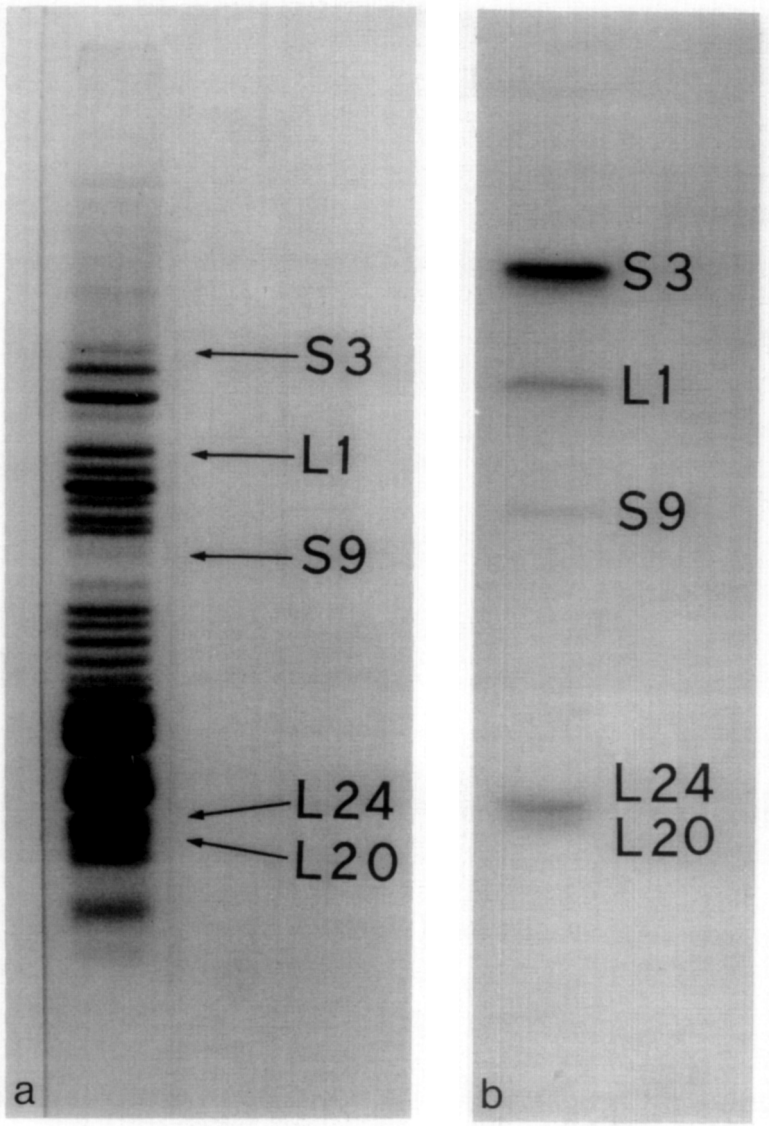

Figure 2. SDS gel electrophoresis of ribosomal proteins labelled in vivo with $^{32}PO_4$, as described in Material and Methods. Electrophoresis was carried out in 12-16% acrylamide gradients according to Laemmli (20) A: Photograph of the gel. B: Autoradiogram.

The relative distribution of radioactivity among the five proteins was also determined (Table 1). It can be seen that protein S3 accounted for the major part of the total radioactivity (70%) and that it was located in the small subunit. It may be

TABLE 1

Ribosomal proteins phosphorylated in vivo

Protein	Molecular weight	% total radioactivity
L20	15,000	3.5
L24	15,500	16.5
S9	32,000	3
L17	42,000	5
S3	55,000	70

Cells were labelled with $^{32}PO_4$ *in vivo* and ribosomal proteins were prepared and analyzed as described in Material and Methods. Numbers in parenthesis refer to numeration of proteins from purified subunits (14).

related to protein S6 of rat liver (2) although its molecular weight is definitely higher (55,000 vs. 36,000). However, its function in ribosome activity may be equivalent. The three acidic proteins belonged to the large subunit, and except for L24, were weakly labelled. The protein S9 had a molecular weight similar to the rate liver ribosomal protein S6. However, the phosphorylation was much less than for protein S3.

These results show that ribosomes from *Physarum polycephalum* are phosphorylated *in vivo*, and further confirm that this phenomenon is general to all eukaryotes. It is of interest to note that this organism is highly primitive and that the phosphorylation of ribosomal proteins has appeared early in evolution. The natural synchrony through the cell cycle and multiple differentiation pathways of *Physarum polycephalum* should make this organism a useful system in which to study this phenomenon further.

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