Phosphorylation of A-proteins by protein kinases bound to yeast ribosomes*

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The ribosomes contain, among many basic proteins, a set of unusually acidic proteins of relative molecular mass 13000. Acidic ribosomal proteins, designated A-proteins, are the only multicopy components of the ribosomal particle. They play a very important role in the interaction of translation factors with ribosomes during protein synthesis [1]. It is interesting that a large pool of A-proteins is found in the cytoplasm. In addition, ribosome-bound A-proteins, but not cytoplasmic ones, become modified by phosphorylation and this modification is required for ribosome activity [2, 3].

It is suggested that the phosphorylation/dephosphorylation mechanism can control the number of active ribosomes in the cell, by regulating the amount of acidic phosphoproteins bound to ribosomal particles. It seems that protein kinases associated with ribosomes can participate in such a regulatory mechanism. Earlier studies have demonstrated that, in many eukaryotic organisms, casein kinase 2 (CK2[1]) is tightly bound to the ribosomes [4–7]. Both in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* two proteins of M_r 13000 and 38000, components of 60S ribosomal subunits, were phosphorylated by protein kinase bound to ribosomes. An enzyme with properties of CK2 was identified in ribosome preparations of either yeast species. In addition, in *S. cerevisiae* another protein kinase with a high substrate specificity toward A-proteins (PK60S) was also identified [8, 9].

The present investigation was undertaken for comparative analysis of the *in situ* protein phosphorylation of ribosomes isolated from the yeast species representing different taxonomic groups.

The Triton X-100 treated ribosomes were used as both an enzyme and a substrate source. It was found (Fig. 1), that the proteins of M_r 13000 and 38000 were phosphorylated only in four of the eleven yeast species tested. These were: *S. cerevisiae, Sch. pombe, Saccharomycodes Ludwigii* and *Trichosporon cutaneum* (Fig. 1a, b, c, k). The highest phosphorylation level was observed in the case of *S. cerevisiae*. It should be pointed out that the level of phosphorylation was clearly dependent on the physiological conditions of the cell growth. It was much lower in the case of *S. cerevisiae* cells grown in anaerobic conditions than in cells grown aerobically (not shown). Endogenous phosphorylation of A-proteins was also much lower in quiescent cells (spores) than in vegetative yeast cells (Fig. 2). This is in agreement with the earlier observations that ribosomes from exponentially growing cells have almost twice as much of A-proteins as ribosomes from the stationary phase cultures; similarly, ribosomal particles in polysomes contain more of acidic proteins than free ribosomes [10].

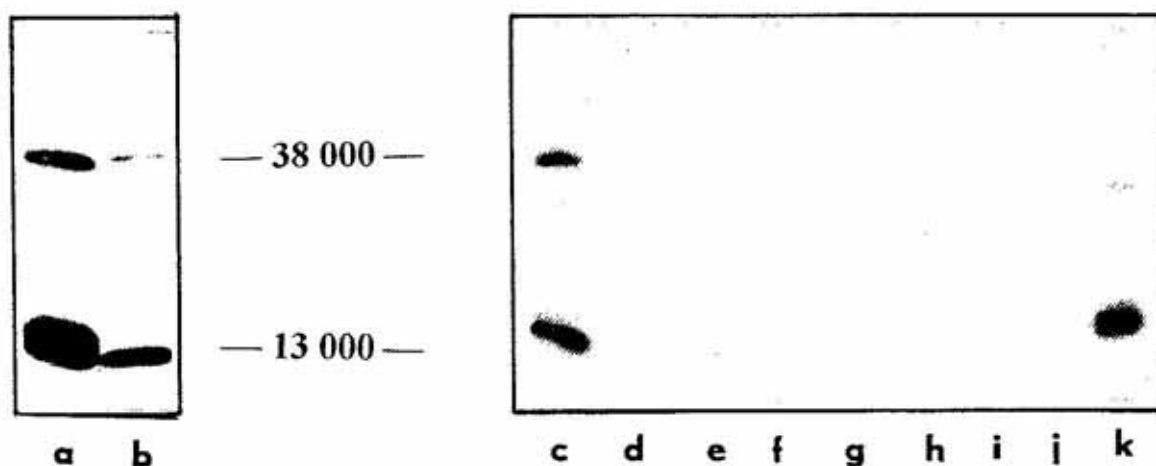[1]Abbreviations: CK2, casein kinase 2; *S., Saccharomyces; Sch., Schizosaccharomyces*

Fig. 1. Endogenous phosphorylation of ribosomes from different yeast species.
Lanes: a, *S. cerevisiae*; b, *Schizosaccharomyces pombe*; c, *Saccharomycodes Ludwigii*; d, *Pichia stipitis*; e, *Fabospora fragilis*; f, *Kluyveromyces fragilis*; g, *Kloeckera apiculata*; h, *Candida utilis*; i, *Rhodotorula gracilis*; j, *Torulopsis utilis*; k, *Trichosporon cutaneum*. Triton X-100 washed ribosomes (75 µg) were incubated with $[\gamma^{32}P]ATP$ under conditions described in [9], then subjected to SDS/polyacrylamide gel electrophoresis and autoradiography.
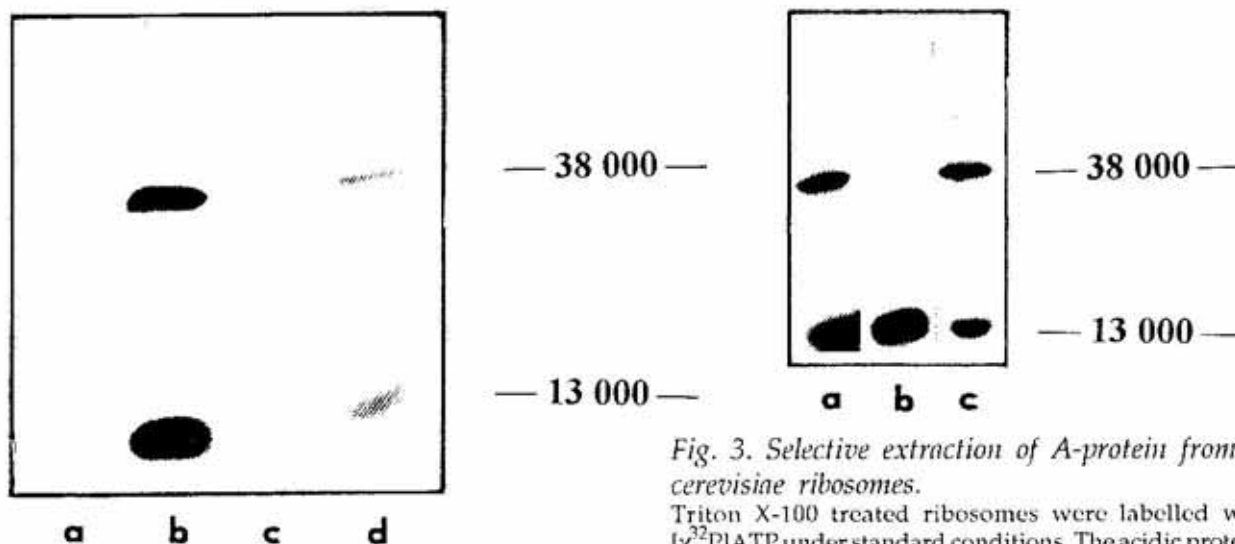

Fig. 2. Endogenous phosphorylation of ribosomes from vegetative (a, b) and quiescent cells (c, d) of S. cerevisiae.
The conditions of reaction and electrophoresis as described in Fig. 1. Lanes: a, c, crude ribosomes; lanes: b, d, Triton X-100 treated ribosomes.

Fig. 3. Selective extraction of A-protein from S. cerevisiae ribosomes.
Triton X-100 treated ribosomes were labelled with $[\gamma^{32}P]ATP$ under standard conditions. The acidic proteins were extracted with 0.25 M NH_4Cl/30% ethanol. Phosphoproteins were detected by SDS/PAGE and subsequent autoradiography. Lanes: a, endogenous phosphorylation of ribosomes; b, protein extracted with 0.25 M NH_4Cl/50% ethanol; c, ribosomes washed with 0.25 M NH_4Cl/50% ethanol (ribosomal "core").

Fig. 4. Isoelectrofocusing of acidic ribosomal proteins from yeast.
The acidic proteins were extracted from 1 mg of Triton X-100 washed ribosomes by 0.25 M NH_4Cl/50% ethanol after being labelled *in vitro* with $[\gamma^{32}P]ATP$. Isoelectrofocusing was performed in 5% polyacrylamide (w/v) slab gels with 6 M urea and 2% LKB ampholines in the 2.5 to 5 pH range [9]. Lanes: a, *S. cerevisiae*; b, *Sch. pombe*; c, *Saccharomycodes Ludwigii*; d, *Trichosporon cutaneum*.
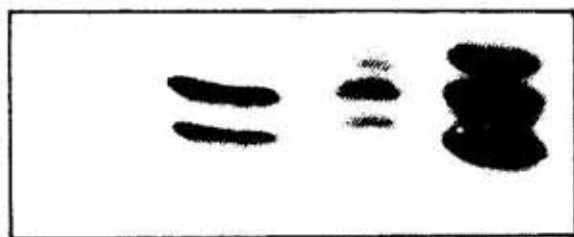
Treatment of the ribosomes with 0.25 M NH4Cl in 50% ethanol, allowed for selective extraction the most acidic ribosomal proteins of M_r 13000. The phosphoprotein of M_r 38000 remained bound to the ribosome (Fig. 3); it is known as AO [11] and belongs to the so called ribosomal "core".

By using isoelectrofocusing in the acidic pH range, endogenously phosphorylated A-proteins extracted with 0.25 M NH4Cl-50% ethanol were resolved into several forms differing in charge. As shown previously [9] in the case of *S. cerevisiae* three different P-forms were observed.

In the fission yeast *Sch. pombe*, four phosphorylated forms of A-proteins were detected on isoelectrofocusing gels (Fig. 4b). On the other hand, isoelectrofocusing analysis of A-proteins extracted from *Saccharomycodes Ludwigii* and *Trichosporon cutaneum* ribosomes (Fig. 4c, d) revealed two forms of different charge. So far, there is no data available concerning genetic and biochemical analysis of these proteins and their physiological role. Protein kinases which are involved in their modification need further examination.

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