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Short Communication

Differential phosphorylation of ribosomal acidic proteins from yeast cell by two endogenous protein kinases: casein kinase-2 and 60S kinase*

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The native 80S ribosomes isolated from *Saccharomyces cerevisiae* (strain W303) cells was phosphorylated by two endogenous protein kinases: multifunctional casein kinase-2 (CK-2) and specific 60S kinase. Three acidic proteins within the 60S ribosomal subunit: YP1β, YP1β′ and YP2α are phosphorylated by both kinases. The other two proteins: YP1α and YP2β are predominantly phosphorylated by CK-2 but not by 60S kinase. This was confirmed in the experiment with the recombinant protein, YP2β, as a substrate, which is practically not phosphorylated by specific 60S kinase. These results together with the previous data based on the target amino-acid sequences suggest that, in addition to the multifunctional casein kinase-2 and specific 60S kinase, there exist probably other protein kinase(s) which phosphorylate the ribosomal acidic proteins in the cell.

The 60S ribosomal subunit from *Saccharomyces cerevisiae* cells contains a set of acidic proteins (P-proteins) playing an important role in the ribosome function [1]. P-proteins are mainly located on the surface of ribosomes and can be extracted from the particles by washing with the solution of 0.3–1.0 M ammonium chloride in 50% ethanol, giving the split protein (SP) fraction and a core particle [2]. The four acidic proteins from the SP fraction were purified, and genetically and chemically characterized. These P-proteins having molecular masses of about 13 kDa [3, 4], are encoded by four independent genes [4–6]. According to the uniform nomenclature, they are named: YP1α, YP1β, YP2α, YP2β [7]. Protein YP1β can be found in some SP preparations also as a truncated polypeptide lacking the first eight amino acids, and that form has been proposed to be called YP1β′ [8].

Ribosomal P-proteins were found in the yeast not only as ribosome-bound polypeptides. A

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large pool of free P-proteins is observed in the cell cytoplasm [9–12]. The amount of acidic proteins present in the ribosomal particles depends on the growth stages of the cell [1, 13]. This means that an exchange of P-proteins between cytoplasm and ribosomes takes place [12, 14]. This process might be mediated by a phosphorylation-dephosphorylation mechanism since P-proteins found in the cytoplasm are unphosphorylated [10, 11] and phosphorylation is required for binding to the particle *in vitro* [3, 15] as well as *in vivo* [16, 17]. Moreover, reconstitution of the active ribosomal particles from the split P-proteins and ribosomal core particles depends on phosphorylation of the acidic proteins. Only the phosphorylated proteins restore to the core particle the capability to bind GDP in the presence of translation factor during the elongation step of protein synthesis [3]. All these data indicate that phosphorylation of the split P-proteins is a mechanism which can regulate a number of active ribosomal particles and, thus, protein synthesis [1].

It is known that 60S ribosomal P-proteins from yeast are phosphorylated *in vitro* by at least two protein kinases: multifunctional casein kinase-2 (CK-2) [18] and highly specific 60S kinase [19]. 60S kinase was in the free form isolated from the cytoplasm of yeast cells [19]. The activity of this enzyme was detected also in the ribosome-bound protein fraction [20]. In contrast to CK-2, 60S kinase shows high specificity for P-proteins: no other protein substrate for 60S kinase has been found. On the basis of these data the question arises whether CK-2 and 60S kinase phosphorylate all four P-proteins or modify selectively only some of them. The aim of the present studies is to answer this question.

MATERIALS AND METHODS

Enzymes. Purified yeast protein kinases CK-2 [18] and 60S kinase [19] were obtained as previously described. One unit kinase activity is defined as the amount of the enzyme required for the incorporation of 1 pmol phosphate from [γ-^{32}P]ATP into substrate/min.

Acidic proteins extraction. P-proteins were extracted from the untreated and alkaline phosphatase treated ribosomes by washing the purified ribosomes with 0.5 M ammonium chloride in 50% ethanol as described by Sanchez-Madrid *et al.* [2].

Electrofocusing. P-proteins (about 4 μg) were resolved by isoelectrofocusing using 2% Pharmacia ampholytes, pH 2.5–5.0 range. 5% polyacrylamide gel slabs (210 mm × 100 mm × 0.8 mm) containing 6 M urea and 0.1% nonionic detergent Tween 20 were used. The isoelectrofocusing was conducted by applying a voltage step gradient for seven periods of 15 min each. The first 15 min of isoelectrofocusing was conducted at 50 V. In the succesive 15 min periods, the voltage was increased by 100 V until it reached 600 V. This took 2 h. Then, the voltage was reduced and isoelectrofocusing was continued at the constant voltage of 250 V for 18 h. Finally, the voltage was increased again up to 600 V for a short time (30 min). The gels were silver stained. The position of the different protein forms is marked.

Phosphorylation of ribosomes in vitro. Highly purified yeast 80S ribosomes (0.5 mg ribosome protein), devoid of endogenous kinase activity, were phosphorylated under standard conditions with CK-2 [18] or 60S kinase [19], with [γ-^{32}P]ATP (Amersham) (500–1000 c.p.m./pmol) and 5 units of either higly purified protein kinase isolated from yeast cells. Assays were terminated by adding an equal volume of the extraction solution containing 1.0 M ammonium chloride in ethanol. The extracted, ^{32}P-labelled acidic proteins were separated by isoelectrofocusing and then detected by autoradiography. Recombinant protein YP2β was a gift from Dr. J.P.G. Ballesta. The assays of ^{32}P-incorporation into the increasing amounts of the recombinant P-protein were performed for both kinases and the samples were prepared and radioactivity counted as described by Pilecki *et al.* [19].

RESULTS AND DISCUSSION

The SP proteins extracted from the purified ribosomes were separated by isoelectrofocusing in a polyacrylamide gel. Up to ten stained protein bands were revealed (Fig. 1A). When the ribosomes were treated with alkaline phosphatase before the extraction, only five bands could be detected in the electrofocused gel. These bands correspond to the non-phospho-

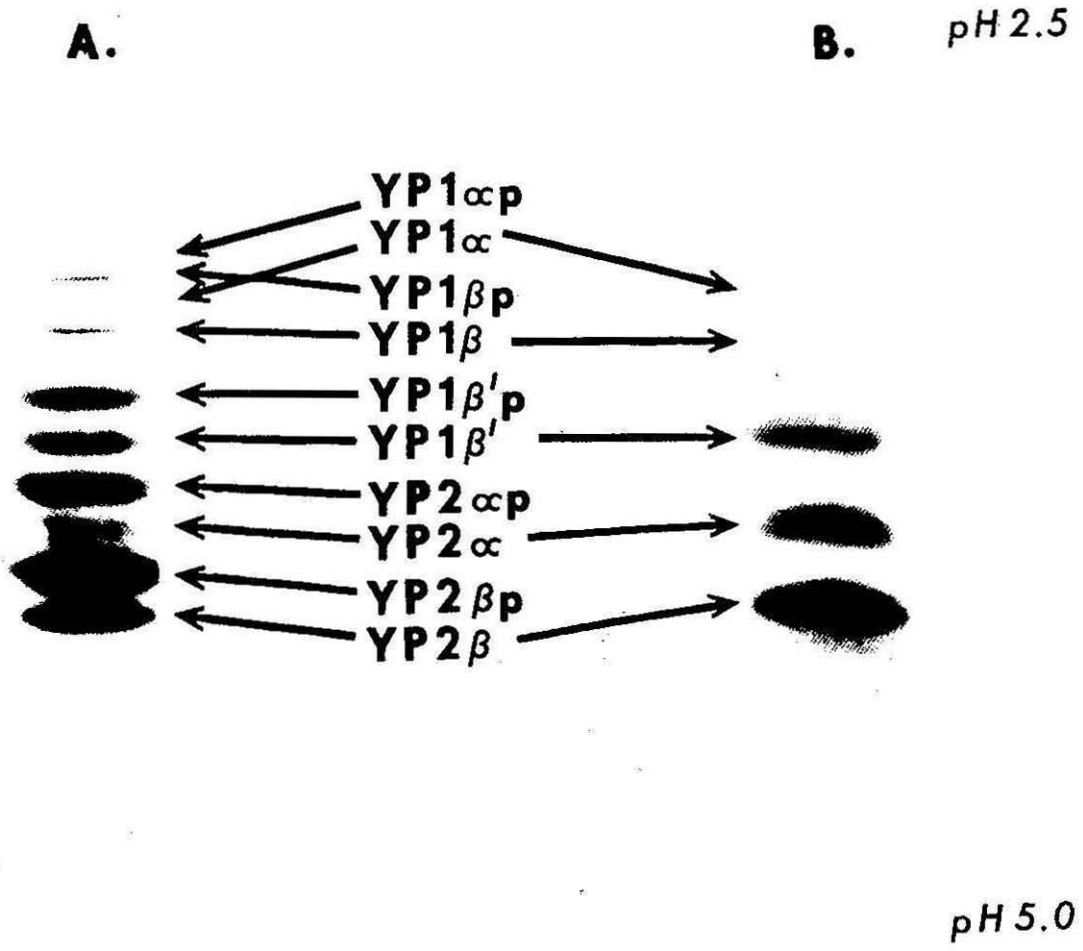

Fig. 1. Separation of yeast P-proteins by isoelectrofocusing in a polyacrylamide gel.
The P-proteins (about 4 μg) were resolved by isoelectrofocusing as described in Methods. The gels were silver stained. The position of the different protein forms is marked.

rylated forms of the proteins (Fig. 1B). Both forms of YP1α protein are present in much lower amounts than the other proteins. Also the native form of YP1β is found in minor amounts while the truncated YP1β' protein form accumulates in this preparation, probably due to proteolytic degradation that takes place during protein preparation under the extraction conditions applied [8].

In further experiments, the same amount of 80S ribosomes was phosphorylated by equivalent amounts of either CK-2 or 60S kinase. Figure 2 presents the autoradiogram of the ribosomal P-proteins extracted from the treated ribosomes and then separated by isoelectrofocusing. In the case of CK-2 treated ribosomes (Fig. 2A), the intensity of radioactive spots is roughly proportional to the amount of the non-phosphorylated form of the proteins in the stained gel. This points to a lack of selectivity of the enzyme for any specific P-protein. On the contrary, in the case of 60S kinase, only three proteins are phosphorylated: YP1β, YP1β' and YP2α (Fig. 2B). The two remaining proteins are phosphorylated by 60S kinase to a negligible extent. These differences are more evident in Table 1 where the ^{32}P incorporation into individual proteins is presented. The very low level of phosphorylation of YP1α and YP2β by 60S kinase is intriguing because the other members of the same protein family: YP1β, YP1β' and YP2α are extensively modified. This selective action of the enzyme may be due either to specific conformation of the proteins when bound to the ribosome structure or the lack of the proper amino-acid sequences surrounding the target serine.

To solve this question, we have used the purified recombinant protein YP2β as a substrate for both protein kinases. As shown in Fig. 3, while CK-2 modifies extensively the recombinant YP2β, 60S kinase does not phosphorylate

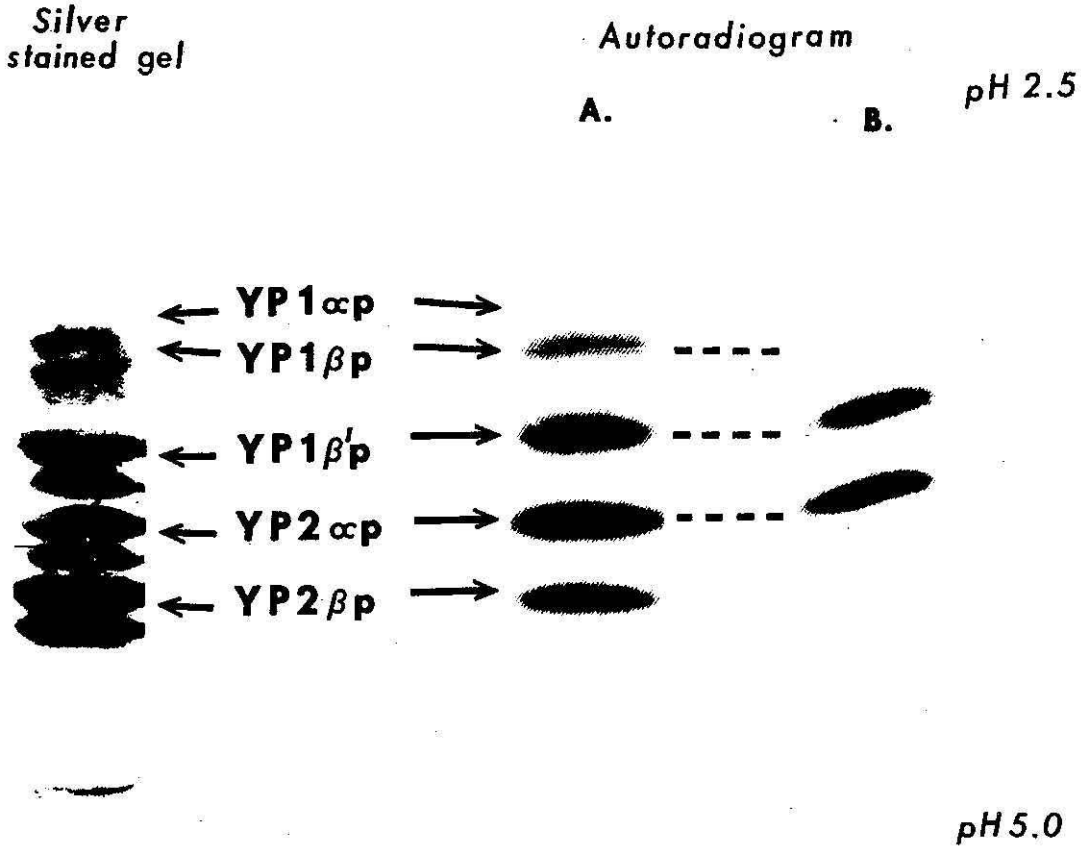

Fig. 2. Autoradiogram of isoelectrofocusing gels of P-proteins phosphorylated in vitro by the casein kinase-2 (A) and 60S kinase (B).

it at any tested substrate/enzyme ratios. These results indicate that phosphorylation of YP2β does not depend on its binding to the ribosome. Thus different effect caused by 60S kinase and CK-2, depends mainly on the primary structure of the polypeptide chain substrate. One of the common features of all eukaryotic ribosomal P-proteins is the occurrence at the C-terminus of polypeptides, of serine residues surrounded by a cluster of glutamic and aspartic acids. In the case of YP2β, this characteristic grouping is located at serine residue in the position 100. The

Table 1

Phosphorylation of ribosomal acidic proteins by CK-2 and 60S kinase.

The stained and autoradiographed gels of acidic proteins phosphorylated either by CK-2 or by 60S kinase were prepared as described in the Methods. [^{32}P]phosphoprotein bands were excised from the gel and the radioactivity was measured in the scintillation counter. The percentage of radioactivity incorporated into individual phosphoproteins was calculated for each of the two experiments separately after ^{32}P-background subtraction.

^{32}P-proteins	CK-2		60S kinase	
	c.p.m.	radioactivity (%)	c.p.m.	radioactivity (%)
YP1αp	1 437	4.3	23	0.1
YP1βp	4 415	13.2	3 039	16.4
YP1β'p	10 759	32.2	7 274	39.3
YP2αp	8 676	26.0	8 022	43.3
YP2βp	8 104	24.2	141	0.7

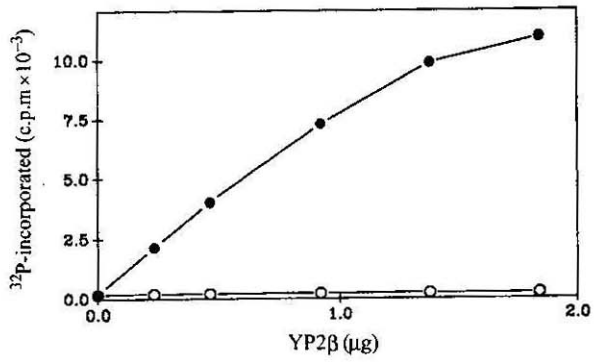

Fig. 3. Incorporation of [γ-^{32}P]ATP into the recombinant protein YP2β.
The assays of ^{32}P-incorporation into increasing amounts of the recombinant P-protein were performed for CK-2 (●) and 60S kinase (○). The samples were prepared and radioactivity counted as described by Pilecki *et al.* [19] and in the Methods.

target residues for CK-2 in the mammalian ribosomal P-proteins were also identified at the C-terminal side of polypeptides [21]. Such an arrangement of acidic amino acids in the polypeptide chain, determines the specific recognition of the substrate by CK-2 [22]. Moreover, synthetic heptapeptides having similar amino -acid sequences have also been used as artificial substrates for kinetic studies on CK-2 and 60S kinase [23]. Both enzymes showed remarkable differences in the kinetic constants: CK-2 exhibited much higher affinity for the tested peptides than did 60S kinase.

Our previous data and the results presented in this paper suggest that the last serine residues located close to C-terminus of all P-polypeptides are probably the target for CK-2 but not for specific 60S kinase. Since it has been previously shown that protein YP2β is phosphorylated *in vivo* at serine 19, near the N-terminus of the polypeptide [16], it can be inferred that probably CK-2 is not the enzyme involved in modification of Ser-19. Moreover, the inability of 60S kinase to modify YP2β strongly suggests that there must be at least one still uncharacterized enzyme, different from CK-2 and 60S kinase, implicated in this function.

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