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QUARTERLY

Subcellular localization of two different type-1 casein kinases from yeast*

Piotr Dukowski[a], Ryszard Szyszka and Józef Bednara[b]

Department of Molecular Biology and [b]Department of Plant Anatomy and Cytology, M. Curie-Skłodowska University, Akademicka 19, 20–033 Lublin, Poland

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Specific antibodies directed against the two yeast type-1 casein kinases (CK1) were used to study the localization of both 45 kDa and 27 kDa casein kinase species in yeast cells by immunofluorescence. Our results indicate that the larger and smaller CK1 species are localised in different compartments of the yeast cell. The 45 kDa enzyme is present in the cytoplasm of the cell both during the logarithmic and stationary growth phase. The 27 kDa CK1 was found in the nucleus in the cells in logarithmic growth phase while the enzyme from the stationary phase was present in the cytoplasm.

Our results suggest that the 27 kDa casein kinase may play some role in yeast cell division control by displacement from the nucleus to the cytoplasm.

Casein kinases are cyclic nucleotide independent protein kinases (E.C. 2.7.1.27) preferring acidic proteins as substrates. They are classified into two broad groups, type-1 and type-2 [1]. Type-1 casein kinases (CK1)[1] have been purified from different sources and they are present everywhere in the animal kingdom [2 - 4]. In yeast, two species of type-1 casein kinases have been described with properties differing from those of the enzymes identified in higher eukaryotes [5, 6, 7]. The activity of yeast CK1 has been found in the cytosol, mitochondria, and microsomes [8]. The eukaryotic nucleus also contains this type of casein kinase [9]. A wide variety of *in vitro* substrates for mammalian casein kinase I have been identified. Among them are the *Simian virus* 40 (SV40) large T antigen [1]; components of the translation apparatus including initiation factors 4B, 4E and 5 and aminoacyl-tRNA synthetases [1, 10, 11]; nonhistone nuclear proteins, including RNA polymerases I and II [12]; and metabolic enzymes, notably glycogen synthetase [1, 13].

Antisera specific against the two yeast type-1 casein kinases (14) were developed and used to investigate the localization of both enzymes in the compartments of yeast cells. Our current studies using immunocytochemical methods indicated that the 45 kDa casein kinase was present in the cytoplasm while the 27 kDa casein kinase was changing its localization by migrating from the nucleus to the cytosol. Most of the 27 kDa enzyme was present in the nuclei of the cells in the logarithmic growth phase, whereas in the stationary phase cells the kinase was displaced to the cytoplasm.

METHODS

Saccharomyces cerevisiae, a diploid strain SKQ2N and a haploid strain ABYS1 [15], with

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[a]To whom correspondence should be addressed

[1]Abbreviations: BSA, bovine serum albumin; CK1, type-1 casein kinases; PBS, phosphate-buffered saline (see Methods); PMSF, phenylmethyl sulphonyl fluoride

a deficiency of proteinases A and B and of carboxypeptidases Y and S, were used in all experiments. The growth and sporulation media and the conditions of yeast cultivation were as described previously [16]. The spores were isolated from the sporulation medium by centrifugation and then purified from the remaining vegetative cells with Zymolyase 100T (Seikagoku Kogyo Co., Japan). Spore germination was carried out as described previously [17].

Purification of CK1. Casein kinases of type-1 were isolated from yeast and purified as described before [5]. In short, the postribosomal supernatant was precipitated with ammonium sulphate at 50% saturation and after overnight dialysis against 20 mM Tris/HCl, pH 7.5, containing 6 mM 2-mercaptoethanol, 0.5 mM EDTA and 0.5 mM PMSF, was chromatographed on a DEAE-cellulose column. The type-1 casein kinase activity found in the flow-through fractions was then chromatographed on P-cellulose and on heparin-Sepharose 4B. The applied procedure allowed for good separation and purification to the stage of near homogeneity of both kinase 1 species.

Casein kinase activity was determined as described earlier [5]. The assay mixture in the final volume of 50 µl contained: 50 mM Tris/HCl, pH 7.5, 70 µg of casein, 6 mM 2-mercaptoethanol, 15 mM $MgCl_2$, and 5 - 10 µl of the enzymatic fraction. The reaction was started by addition of 30 µM [γ-^{32}P]ATP (500 - 1000 c.p.m./pmol), and after 15 min of incubation at 30°C it was terminated by adding 10% trichloroacetic acid. The precipitated material was collected on GF/C glass filters. The filters were washed with 10% trichloroacetic acid, dried and counted in a scintillation counter. In the immunological experiments, the enzymes and IgG samples were preincubated for 30 min in ice in 50 mM Tris/HCl, pH 7.5, 100 mM $MgCl_2$, 6 mM 2-mercaptoethanol in the final volume of 25 µl. Bovine serum albumin (20 µg) was added to stabilise the enzyme activity. After preincubation, the radioactive nucleotide and casein were added to the final volume of 50 µl and the incubation was continued for 15 min at 30°C.

Antibodies against CK1. Purified preparations of the 45 kDa or the 27 kDa casein kinase were pooled and used for immunization. A rabbit (approx. 2 - 3 kg in weight) was injected intradermally at 40 - 50 sites on the back with 0.7 mg of the purified enzyme emulsified in an equal volume of complete Freund's adjuvant. After 4 weeks, the rabbit was boosted with the same amount of the enzyme and adjuvant. Sera were collected one week after the second injection and stored in small portions at –20°C. IgG fractions were prepared by chromatography on DEAE-Sephadex A-50 [18]. Antibodies were purified by the affinity method [19].

Immunofluorescence. For immunofluorescence studies, cells from a *Saccharomyces cerevisiae* culture were harvested at the log or stationary phase. Cells were fixed for 90 min with a fresh solution of 4% paraformaldehyde, washed with 0.1 M potassium phosphate, pH 6.5, and cell walls were digested by incubation of yeast with zymolyase as described before [17]. Yeast spheroplasts were washed three times with the 0.12 M K_2HPO_4, 33 mM citric acid buffer, pH 7.4, supplemented with 1.2 M sorbitol, and then the cell suspension was applied to poly-L-lysine coated slides. The spheroplasts attached to the slides were lysed by washing the slides with methanol for 6 min at –20°C and then with acetone for 30 s. The slides were rinsed in four changes (for 5 min each) of PBS (0.07 M KH_2PO_4, 0.14 M NaCl, 2 mM KCl, pH 7.2), containing 1% BSA. Incubation with the first antibody directed against the casein kinases lasted 60 min at 37°C in a humid chamber. Dilution of the anti-CK1 45 kDa or 27 kDa antibody was 30- to 50-fold in PBS containing 3% BSA. After this step, the cover glasses were washed with PBS containing 1% Triton X-100 and then incubated in the presence of the second antibody (FITC-conjugated goat anti-rabbit serum, Sigma, U.S.A.). Dilution of the second antibody was 50-fold in PBS containing 3% BSA. The incubation lasted for 60 min at 37°C in a humid chamber. The cover glasses were washed with PBS, mounted on microscope slides and embedded in 90% glycerol/PBS. The presence of the FITC-labelled antibody was verified by immunofluorescence microscopy [20].

RESULTS AND DISCUSSION

Two protein kinases of 45 kDa and 27 kDa, preferentially phosphorylating casein and phosvitin in the presence of ATP as a phosphoryl donor, were isolated from yeast. Both ki-

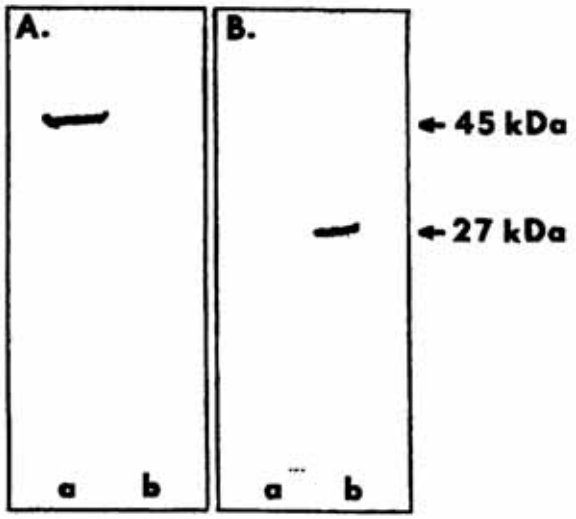

Fig. 1. Specificity of polyclonal antibodies against yeast type-1 casein kinases.
CK1 45 kDa, 0.5 µg (a), and CK1 27 kDa, 0.5 µg (b) were separated on 10% SDS/PAGE [21], blotted on nitrocellulose paper and immunostained with purified: A, anti-CK1 45 kDa serum; or B, anti-CK1 27 kDa serum.

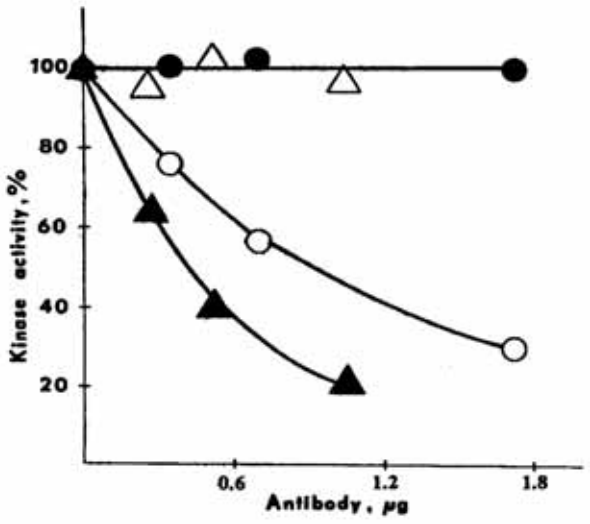

Fig. 2. Inhibition of type-1 casein kinase activities by the IgG fraction.
The 45 kDa casein kinase (open symbols) or 27 kDa casein kinase (closed symbols) was preincubated with increasing concentrations of the IgG fractions isolated from the serum after immunization with the 45 kDa enzyme (circles) or with the 27 kDa enzyme (triangles). The enzymatic reaction was initiated by addition of casein and [γ-^{32}P]ATP as described under Methods.

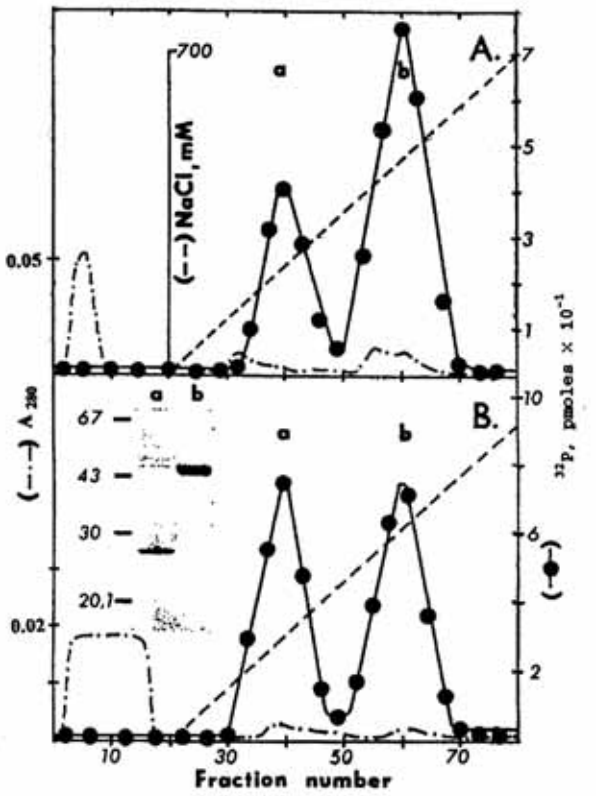

Fig. 3. Heparin-Sepharose chromatography of yeast type-1 casein kinases isolated from the cytosol of the logarithmic (A) and stationary (B) growth phase cells.
An enzyme sample (8 - 10 µg of the protein), after P-cellulose chromatography, was applied to a heparin-Sepharose 4B column (0.8 × 9 cm), equilibrated with 20 mM Tris/HCl, pH 7.5, containing 6 mM 2-mercaptoethanol, 0.5 mM EDTA, and 0.5 mM PMSF. The protein was eluted with a linear 0 - 0.7 M NaCl gradient in this buffer. Fractions of 2 ml were collected and 5 µl aliquots of each were assayed in the presence of casein (●). The insert in part B represents the SDS/PAGE of kinase preparations: a) 27 kDa casein kinase; b) 45 kDa casein kinase. The numbers on the left of panel B represent molecular masses in kilodaltons.

nases displayed practically the same substrate specificity except that the 27 kDa enzyme phosphorylated, in some protein substrates, threonine residues instead of serine residues. Antibodies against both type-1 casein kinases were isolated. Figure 1 shows an immunoblot of the purified type-1 casein kinases with the antibodies raised against these enzymes. The casein kinase 45 kDa antibody recognizes only the polypeptide of molecular mass 45 kDa, but not the 27 kDa protein (Fig. 1A). When the antibody against the casein kinase of 27 kDa

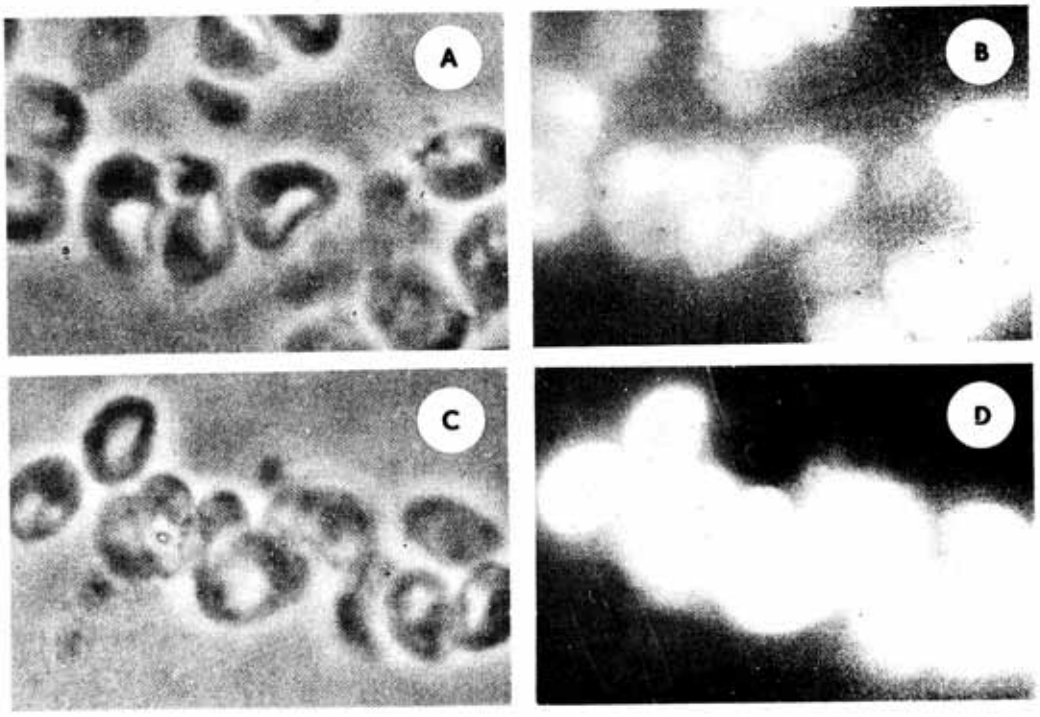

Fig. 4. Immunofluorescence staining of the logarithmic growth phase yeast cells with type-1 casein kinase antibodies.
The cells were stained by indirect immunofluorescence using either (B) affinity-purified anti-CK1 27 kDa serum (dilution 30-fold) or (D) anti-CK1 45 kDa serum (dilution 50-fold). (A) and (C) show the corresponding phase contrast images.

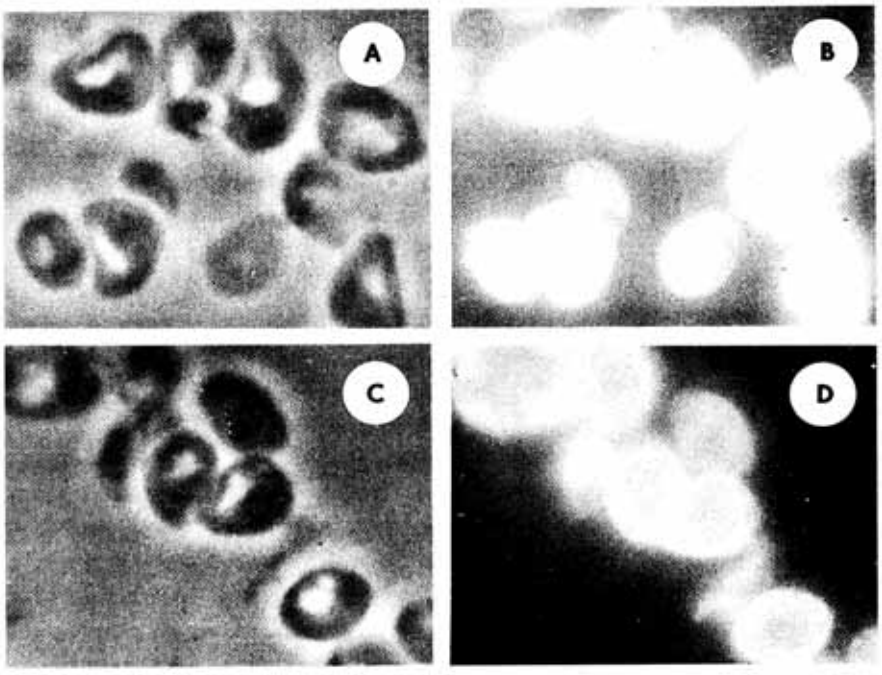

Fig. 5. Immunofluorescence staining of the stationary growth phase yeast cells with type-1 casein kinase antibodies.
The cells were stained by indirect immunofluorescence using either (B) affinity-purified anti-CK1 27 kDa serum (dilution 30-fold) or (D) anti-CK1 45 kDa serum (dilution 50-fold). (A) and (C) show the corresponding phase contrast images.

was used, it crossreacted only with the 27 kDa polypeptide (Fig. 1B). This result may suggest immunological distinctiveness of the two yeast type-1 casein kinases.

The effect of the two antibodies on the activity of the casein kinases was tested (Fig. 2). The antibodies against the 45 kDa and 27 kDa kinases inhibited only the respective enzymes.

The quantitative proportion between the two investigated purified enzymes was examined (Fig. 3). In the cytosol of the cells from the logarithmic growth phase the ratio of the 27 kDa to 45 kDa casein kinase was 1:3 (Fig. 3A). This relation was changed to 1:1 in the cytosol of the stationary growth phase cells (Fig. 3B).

To answer the question whether the 45 kDa and 27 kDa yeast protein kinases exhibit an identical subcellular localization, immunofluorescence studies were performed. Rabbit anti-yeast type-1 casein kinase antisera and spheroplasts obtained from the logarithmic (Fig. 4) and stationary (Fig. 5) growth phase yeast cells were used for these experiments.

Phase contrast microscopy images are shown in Figs. 4D and 5D. Cells stained with the anti-45 kDa CK1 antibody showed fluorescence in the cytoplasm. Figures 4B and 5B show immunofluorescence staining of yeast cells with the anti-27 kDa CK1 antibodies. Most of the 27 kDa kinase was found in the nucleus of yeast cells at the logarithmic phase of growth (Fig. 4B), whereas the stationary growth phase cells showed fluorescence signals in the cytoplasm (Fig. 5B).

The results shown here indicate that both yeast type-1 casein kinases are detected in particular cell fractions. The 27 kDa casein kinase has been identified in the cytoplasm and nucleus whereas the 45 kDa enzyme was present only in the cytoplasm of yeast cells. Previously CKII has been shown to be localized in both those cell compartments [1, 22 - 24]. CK1 has been detected only in the nucleus [1, 9].

Our results suggest that translocation of the 27 kDa CK1 from the nucleus to the cytoplasm during transition of the cells from the logarithmic to the stationary phase of growth may be related to regulation of the transcription process. More work is needed to elucidate the function of yeast CK1 during differentiation of *S. cerevisiae*.

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