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CK2 activity is modulated by growth rate in *Saccharomyces cerevisiae*

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ABSTRACT

CK2 is a highly conserved protein kinase controlling different cellular processes. It shows a higher activity in proliferating mammalian cells, in various types of cancer cell lines and tumors. The findings presented herein provide the first evidence of an *in vivo* modulation of CK2 activity, dependent on growth rate, in *Saccharomyces cerevisiae*. In fact, CK2 activity, assayed on nuclear extracts, is shown to increase in exponential growing batch cultures at faster growth rate, while localization of catalytic and regulatory subunits is not nutritionally modulated. Differences in intracellular CK2 activity of glucose- and ethanol-grown cells appear to depend on both increase in molecule number and k_{cat}. Also in chemostat cultures nuclear CK2 activity is higher in faster growing cells providing the first unequivocal demonstration that growth rate itself can affect CK2 activity in a eukaryotic organism.

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1. Introduction

Protein kinase CK2 is a highly conserved, essential protein kinase [1], which phosphorylates more than 300 substrates, involved in transcription, translation, signal transduction, survival and cell cycle [2]. It is a tetrameric enzyme, composed of two catalytic subunits (α and α′) and two regulatory subunits (β), which enhance CK2 stability and activity and modulate its substrate selectivity [3]; however, recent evidences indicate that individual β subunits may have CK2-independent functions as well [4]. In *Saccharomyces cerevisiae* the two catalytic subunits, α and α′ are encoded by *CKA1* and *CKA2* genes and the two regulatory subunits, β and β′, by *CKB1* and *CKB2* genes. Genetic studies in yeast demonstrated that CK2 is essential for cell viability [5]. The two catalytic subunits may have non-redundant roles, CK2α being primarily involved in the maintenance of cell polarity [6] and CK2α′ in cell-cycle regulation [7].

CK2 is a constitutively active enzyme, independent of second messengers [2]; yet, a number of distinct mechanisms contribute to its modulation, such as regulated expression and assembly, post-translational modifications, protein–protein interactions, regulation by natural compounds (heparin, polyamines, etc.) [4]. CK2 is also regulated by a tight modulation of its subcellular localization, since it exerts different functions in the various cellular compartments [8]. In particular, CK2 subunits often show a nuclear localization [9,10], which in mammalian cells is reported to increase after serum addition and in tumor cells [11,12], thus appearing to be linked to active cell proliferation. In keeping with these observations, CK2 activity is higher after hormone or growth factor stimulation [13,14]. Besides, abnormally elevated CK2 activity is observed in various types of cancer and cancer cell lines [15,16]. CK2 also exhibits oncogenic activity when overexpressed and shows cooperativity when combined with several oncogenes [16]. In accordance with the emerging view of CK2 as a cancer marker and a putative new therapeutic target [16,17], a positive correlation between CK2 activity and cellular proliferation rate has been suggested [15,18]. By using *S. cerevisiae* as a model, we test this hypothesis by dissecting the effect of growth rate and nutrient sensing and metabolism on CK2 activity through the use of chemostat-grown yeast cultures. Our results show, for the first time, that CK2 activity is directly correlated to growth rate and not to the carbon source.

2. Materials and methods

2.1. Yeast strains and growth conditions

Saccharomyces cerevisiae strains used in this study are listed in Table 1. Batch culture were grown in synthetic complete media, prepared by assembling the carbon source (2% glucose, 2% ethanol, 2% raffinose or 3% glycerol), 6.7 g/L yeast nitrogen base and complete supplemented mixture (CSM, MP Biomedials).

2.2. Protein extraction and Western blotting

Crude protein extracts were obtained by standard glass beads method using lysis buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl,

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Table 1
Yeast strains used in this study.

Yeast strain	Genotype	Source
BY4741	MATa his3_1 leu2_0 met15_0 ura3_0	Open BioSystem
CKA1-TAP	MATa his3_1 leu2_0 met15_0 ura3_0 CKA1-TAP	Open BioSystem
CKA2-TAP	MATa his3_1 leu2_0 met15_0 ura3_0 CKA2-TAP	Open BioSystem
CKB1-TAP	MATa his3_1 leu2_0 met15_0 ura3_0 CKB1-TAP	Open BioSystem
CKB2-TAP	MATa his3_1 leu2_0 met15_0 ura3_0 CKB2-TAP	Open BioSystem
cka1Δ	MATa his3_1 leu2_0 met15_0 ura3_0 cka1::kanMX4	Euroscarf
cka2Δ	MATa his3_1 leu2_0 met15_0 ura3_0 cka2::kanMX4	Euroscarf
CEN.PK113-7D	MAT a MAL2-8 c SUC2 L.	Brambilla collection

0.1% NP-40, 10% glycerol) plus 1 mM PMSF (phenylmethanesulphonylfluoride), protease inhibitor mix (Complete EDTA free protease inhibitor cocktail tablets, Roche) and phosphatase inhibitor mix (Sigma). When indicated, protein extracts were dialysed against 50 mM Tris–HCl pH 7.5, 150 mM NaCl, 0.1% NP-40, 10% glycerol, 1 mM PMSF, 1 mM Na_3VO_4, using dialysis tubes with a cut-off of 12,000 Da. Nuclear and cytoplasmic extracts were obtained using NE-PER Extraction kit (Pierce Biotechnology) on spheroplasts, as reported in [19]. Protein concentration was determined using the Bio-Rad protein assay. Western blot analysis was performed using anti-TAP monoclonal antibody (1:2500 dilution, Open Biosystems), anti-Nop1 antibody as nuclear control (1:5000 dilution, EnCor Biotechnology) and anti-Cdc34 polyclonal antibody as loading control (1:1000 dilution).

2.3. CK2 activity towards peptide substrates

Crude protein extracts from yeast cells were obtained as reported above, using lysis buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 0.1% NP-40, 10% glycerol) plus 1 mM DTT (dithiothreitol), protease inhibitor mix (Complete EDTA free protease inhibitor cocktail tablets, Roche) and phosphatase inhibitor mix (Sigma). CK2 activity was tested on the indicated amount of crude protein extracts or of nuclear protein extracts in a medium containing 50 mM Tris–HCl buffer (pH 7.5), 10 mM $MgCl_2$, 100 mM NaCl and 100 μM [γ-^{33}P]ATP (specific radioactivity 1000–2000 cpm/pmol) in the presence of the specific peptide substrate RRRADDSDDDDD (250 μM unless otherwise specified, [20]) or of the eIF2β-derived peptide (MSGDEMIFDPTMSKKKKKKKKP, 250 μM [21]), where indicated. TBB (4,5,6,7-tetrabromobenzotriazole) (10 μM) was used as selective CK2 inhibitor in control reactions. Assays were carried out in a 30 μl volume at 30 °C and stopped after 10 min of incubation by spotting onto phosphocellulose filters and cooling in ice. Filters were washed in 75 mM phosphoric acid four times and dried before counting. Initial rate data were fitted to the Michaelis–Menten equation and V_{max} and K_m values were determined from Lineweaver–Burk plots.

2.4. CK2 activity towards recombinant His_6-Sic1

His_6-Sic1 was expressed and purified from *Escherichia coli* as previously reported [22]. CK2 activity in crude protein extracts (0.75 μg) towards the recombinant His_6-Sic1 (6 μg) was tested in a reaction mix containing 10 mM Tris–HCl pH 7.5, 150 mM NaCl, 10 mM $MgCl_2$, 1 mM ATP at 30 °C. CK2 phosphorylation was analyzed by SDS–PAGE and blotting with anti-Sic1-pSer201 antibody (dilution 1:2000), and with anti-His probe antibody (dilution 1:1000) as control.

2.5. FACS analysis

Flow cytometric analysis to assay protein content was performed as previously described on a BD Biosciences FACScan [22].

2.6. Chemostat cultivation

Chemostat cultivations were performed as reported [23]. For all the experiments glucose concentration in the reservoir was 5 g l^{-1}. Steady state was achieved after at least six volume changes had passed through and no oscillations had occurred. Each experiment has been run at least in double with a carbon recovery >95%. In each condition cells and carbon dioxide were the only products.

2.7. Statistical analysis

Experiments were carried out in triplicate and repeated at least three times. Results are expressed as means ± SD. Results were compared using the two-sided Student's *t*-test. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. CK2 activity is detectable in nuclear fraction and is modulated by carbon source

To investigate whether CK2 activity was modulated by growth conditions in yeast cells, we first analyzed localizations of the catalytic and regulatory subunits in cells growing in different nutritional conditions. Strains expressing one of different TAP-tagged CK2 subunits (α, α′, β, β′) were grown in glucose or ethanol containing medium, harvested in mid-exponential phase, and localization of the four CK2 subunits were analysed by Western blot using anti-TAP antibody. Our results showed that catalytic and regulatory subunits were only detectable in the nuclear fractions in both conditions (Fig. 1A), confirming the genome-wide data indicating that all four GFP-fused CK2 subunits are mostly localized in the nucleus of glucose growing yeast cells [24]. Moreover, we extend previously reported data by indicating that CK2 nuclear localization is detected also in ethanol growing cells.

Since CK2 is a nuclear enzyme (Fig. 1A), in order to avoid interferences due to the presence of cytoplasmic components, nuclear fractions were isolated from cells growing on glucose or ethanol and CK2 activity was tested. CK2 activity was first determined using the peptide substrate RRRADDSDDDDD (as detailed in Experimental procedures), which is the most commonly used specific substrate for CK2 [20]. In keeping with subcellular localization (Fig. 1A), CK2 activity was undetectable in cytoplasmic extracts (data not shown). Moreover, CK2 activity, assayed as phosphorylation of the synthetic peptide RRRADDSDDDDD, was higher in nuclear extract from glucose growing cells than from ethanol growing ones (Fig. 1B). Specificity of the assay was confirmed by the strong inhibition exerted by the addition of the selective CK2 inhibitor TBB (data not shown) [25]. A more detailed analysis where CK2 kinetic parameters (i.e. K_m and V_{max}) were determined varying the concentrations of the specific peptide, showed no significant differences in K_m, while V_{max} was approximately 1.4-fold higher in glucose growing cells (Fig. 1C).

We previously reported that Sic1, the cyclin-dependent kinase inhibitor, is phosphorylated on Ser201 by CK2 [22,26]. We developed a new *in vitro* CK2 assay using His_6-Sic1 purified from *E. coli* as a CK2 substrate and anti-pSer201-Sic1 antibody to detect phosphorylation. Also this assay showed that CK2 activity is stronger in nuclear extracts prepared from glucose growing cells than from ethanol growing ones (Fig. 1D). These data confirm and ex-

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