Biochemical and Biophysical Research Communications 398 (2010) 44–50

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

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

Farida Tripodi [a,1], Claudia Cirulli [a,1], Veronica Reghellin [a], Oriano Marin [b], Luca Brambilla [a], Maria Patrizia Schiappelli [b], Danilo Porro [a], Marco Vanoni [a], Lilia Alberghina [a], Paola Coccetti [a,*]

[a] Dipartimento di Biotecnologie e Bioscienze, Università di Milano-Bicocca, Piazza della Scienza 2, 20126 Milano, Italy
[b] Dipartimento di Chimica Biologica, Università di Padova, Viale G. Colombo 3, 35121 Padova, Italy

ARTICLE INFO

Article history:
Received 4 June 2010
Available online 17 June 2010

Keywords:
Saccharomyces cerevisiae
Growth rate
Carbon source
Phosphorylation
CK2 activity
Sic1

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.

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,

* Corresponding author. Fax: +39 0264483519.
E-mail address: paola.coccetti@unimib.it (P. Coccetti).
[1] These authors equally contributed to this work.

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.06.028

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 $p < 0.05$.

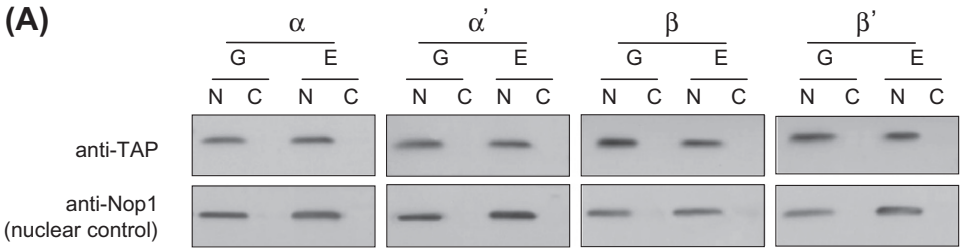
3. Results

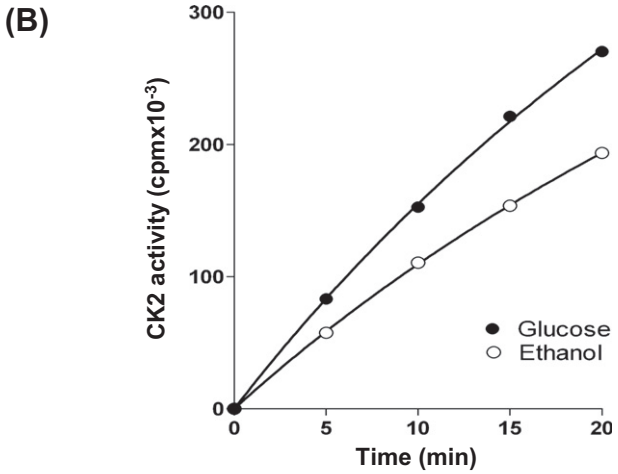
3.1. CK2 activity is detectable in nuclear fraction and is modulated by carbon source

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).

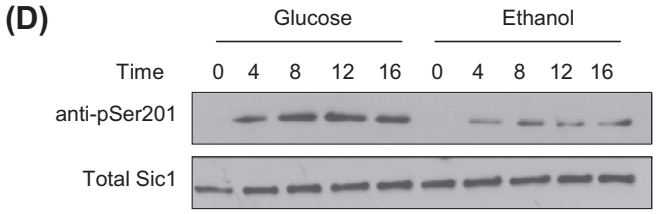
We previously reported that Sic1, the cyclin-dependent kinase inhibitor, is phosphorylated on Ser201 by CK2 [22,26]. We developed a new *in vitro* CK2 assay using His_6-Sic1 purified from *E. coli* as a CK2 substrate and anti-pSer201-Sic1 antibody to detect phosphorylation. Also this assay showed that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





(C)

	Glucose	Ethanol
Km (μM)	67	69
Vmax (pmol/min/mg)	4657	3313



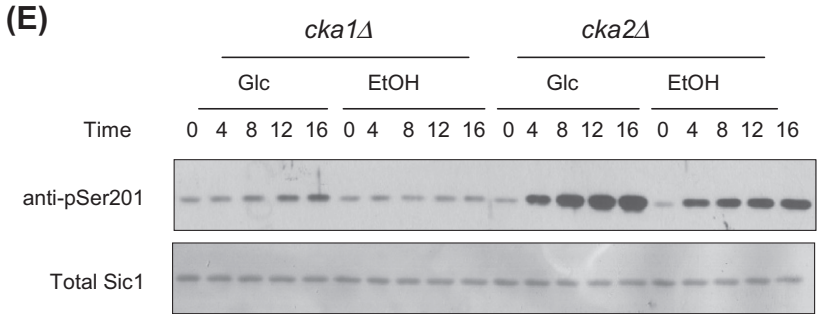


Fig. 1. CK2 activity is modulated by carbon source. (A) Strains expressing TAP-tagged CK2 subunits (α, α′, β, β′) were grown in medium containing glucose or ethanol as carbon source. Samples were taken in mid-exponential phase to compare the subcellular localization of CK2 subunits by western analysis using anti-TAP antibody (anti-Nop1 antibody was used as nuclear marker). One of three independent experiments is reported. (B) 1.5 μg of nuclear protein extracts from cells growing in glucose or ethanol containing media were used to assay CK2 activity towards the specific peptide RRRADDSDDDDD as a function of time and (C) to estimate kinetic parameters (K_m and V_{max}). One of two independent experiments is presented. (D) CK2 activity on nuclear extracts from cells growing in glucose or ethanol containing media were assayed towards the recombinant protein His_6-Sic1. Samples were taken at the indicated time points during the *in vitro* reaction. (E) *cka1Δ* or *cka2Δ* strains were grown in glucose or ethanol containing media, and CK2 nuclear activity was assayed towards the recombinant protein His_6-Sic1 (0, 4, 8, 12, 16 min).

tend results obtained using the synthetic peptide (Fig. 1B) and demonstrate the usefulness of our assay to detect quali–quantitative differences in CK2 activity, employing a physiological substrate instead of a small peptide.

The activity of the two catalytic subunits was separately investigated, measuring CK2 activity from yeast strains expressing only α or α′ subunit. Cells bearing a deletion in one of the two genes encoding the two catalytic subunits (*cka1Δ* or *cka2Δ*) were grown in glucose or ethanol supplemented media, and nuclear CK2 activity was tested using His_6-Sic1 as a substrate. Both *cka1Δ* or *cka2Δ* strains exhibited a higher CK2 activity in glucose than in ethanol growing cells, indicating that (i) activity of both subunits is

modulated by the carbon source and (ii) His_6-Sic1 is prevalently phosphorylated by α subunit (Fig. 1E).

3.2. CK2 activity is modulated by nutritional conditions and correlates with growth rate

Results described above (Fig. 1C and D) suggested that CK2 activity is higher in cells with higher growth rate (*i.e.* glucose growing cells) than in cells with lower growth rate (*i.e.* ethanol growing cells), in keeping with data obtained in mammalian cells correlating CK2 activity with proliferation rate [15,18]. Since growth rate can be easily modulated in yeast cells by changing growth conditions, we analysed cells grown under five different nutritional conditions (complete synthetic media containing glucose, raffinose, ethanol or glycerol as a carbon source and rich medium). Mass duplication time (MDT), protein content (P) and CK2 activity towards the specific substrate peptide RRRADDSD DDDD, were measured for each culture. CK2 activity is directly proportional to the growth rate, being higher in faster growing cells (*i.e.* complete synthetic and rich media) (Fig. 2A and B). Under different nutritional conditions (Fig. 2), yeast cells reprogram their metabolism and concurrently grow with different MDT. To separate the growth rate from metabolic effects, we took advantage of continuous cultures where the growth rate can be set by varying the dilution rate (*i.e.* the flow feed rate) without changing the carbon source [27]. When the dilution rate is kept below a critical value (D_{crit}), glucose-limited yeast cells adopt a fully respiratory metabolism. We have grown the prototrophic strain CEN.PK113.7D in glucose-limited chemostat cultures imposing two dilution rates, $D = 0.23\ h^{-1}$ and $D = 0.1\ h^{-1}$, well below the D_{crit}, that for this strain is $0.28\ h^{-1}$ [28]. Several parameters determined in both conditions (Fig. 3A) all confirmed a fully respiratory metabolism: biomass

(A)

Growth condition	Mass Duplication Time MDT (min)	Protein Content (arbitrary units)	CK2 activity (cpm)
YPD	80 ± 0,7	394 ± 4,5	2,04E+05 ± 1,03E+04
Glc	83 ± 2	403 ± 2,5	2,20E+05 ± 1,08E+04
Raf	96 ± 3,8	302 ± 1,6	1,93E+05 ± 9,82E+03
EtOH	183 ± 13,6	271 ± 2,1	1,60E+05 ± 2,84E+03
Glyc	330 ± 2,1	274 ± 10,2	1,87E+05 ± 1,97E+03

(B)

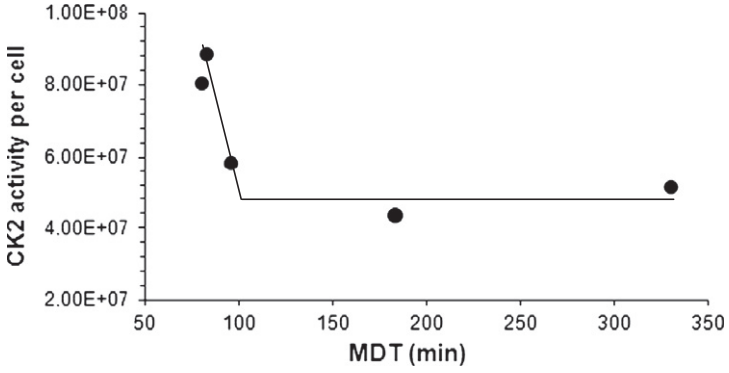


Fig. 2. CK2 activity is modulated by nutritional conditions. Cells were grown in the following media: YPD (rich medium), or complete synthetic medium containing 2% glucose, 2% raffinose, 2% ethanol or 3% glycerol as carbon source. (A) Protein extracts from cells grown in these conditions were used to assay MDT (mass duplication time), total protein content by FACS analysis (P) and CK2 activity towards the specific peptide RRRAADSDDDDD. Means ± standard deviations are indicated ($p < 0.05$). (B) Correlation between MDT and CK2 activity per cell is shown.

(A)

Dilution rate D (h^{-1})	Mass Duplication Time MDT (min)	Carbon source	Yield $Y_{X/S}$	Carbon Recovery (%)	Protein content (arbitrary units)
0,23	180	glucose	0,504	94,8	350
0,10	414	glucose	0,510	98,9	339,5

(B)

	0,23 h^{-1} G	0,1 h^{-1} G
Km (μM)	79	74
Vmax (pmol/min/mg)	7231	4165

Fig. 3. CK2 activity correlates with growth rate. (A) Growth parameters of *S. cerevisiae* CEN.PK113-7D strain in chemostat cultures. Data are the average of at least two experiments. Values for biomass yield ($Y_{S/X}$, gram of cells per gram of carbon source) measured are very close to the theoretic ones (0.51) and are consistent with a fully respiratory metabolism. Carbon recovery is the percentage of the fed carbon found in the products assayed. (B) 0.5 μg of nuclear protein extracts from cells in (A) were used to estimate kinetic parameters towards the specific peptide RRRADDSDDDDD. CK2 activity was assayed with varying concentrations of peptide. Kinetic results obtained in this study are listed. One of two independent experiments is presented.

yield ($Y_{X/S}$) was close to 0.51 g g^{-1} (the expected value for fully respiratory metabolism), respiratory quotient was close to 1 (data not shown), high carbon recovery values were against the presence of products other than biomass or CO_2, ethanol and acetate were absent (data not shown). CK2 kinetic parameters (i.e. K_m and V_{max}) were determined on nuclear extracts from glucose-limited chemostat cultures. Varying the concentrations of the specific peptide RRRADDSDDDDD, we calculated kinetic parameters for CK2 present in nuclear extracts prepared from cell grown at $D = 0.23$ h^{-1} and $D = 0.1$ h^{-1} (Fig. 3B). We found no significant differences in K_m, while V_{max} was approximately 1.5-fold higher in cells with $D = 0.23$ h^{-1}, than in cells with $D = 0.1$ h^{-1}. These data suggest that in glucose-limited chemostat cultures CK2 activity is modulated by the growth rate.

3.3. CK2 activity modulation is not due to small molecules or to a different assembly of the enzyme

Differences in CK2 activity between cell cultures growing with different MDT could be due to multiple factors. Many evidences suggest that CK2 can be modulated by small molecules, which can act as inhibitors or activators of CK2 kinetic activity [4]. To investigate whether this could be the case, we dialysed protein extracts from glucose and ethanol growing cells against fresh buffer, to completely remove small-molecular-weight components, and CK2 activity was tested on the synthetic peptide RRRADDSDDDDD on extracts before and after dialysis (Fig. 4A). The dialysis did not alter CK2 activity, which was always higher in cells growing on glucose than on ethanol (Fig. 4A), indicating that the observed difference was not due to a small activator or inhibitor present in the extracts.

We then reasoned that, although the localization of the four CK2 subunits was the same in glucose and ethanol growing cells, the ratio tetramer vs. free catalytic subunits could be different in the two conditions. Thus we assayed CK2 activity towards the peptide RRRADDSDDDDD (which can be phosphorylated both by the holoenzyme and by free catalytic subunits) and towards the eIF2β-derived peptide (MSGDEMIFDPTMSKKKKKKKKP, which can be phosphorylated only by the holoenzyme [21]). CK2 activity from glucose growing cells was about 1.6-fold higher than from ethanol

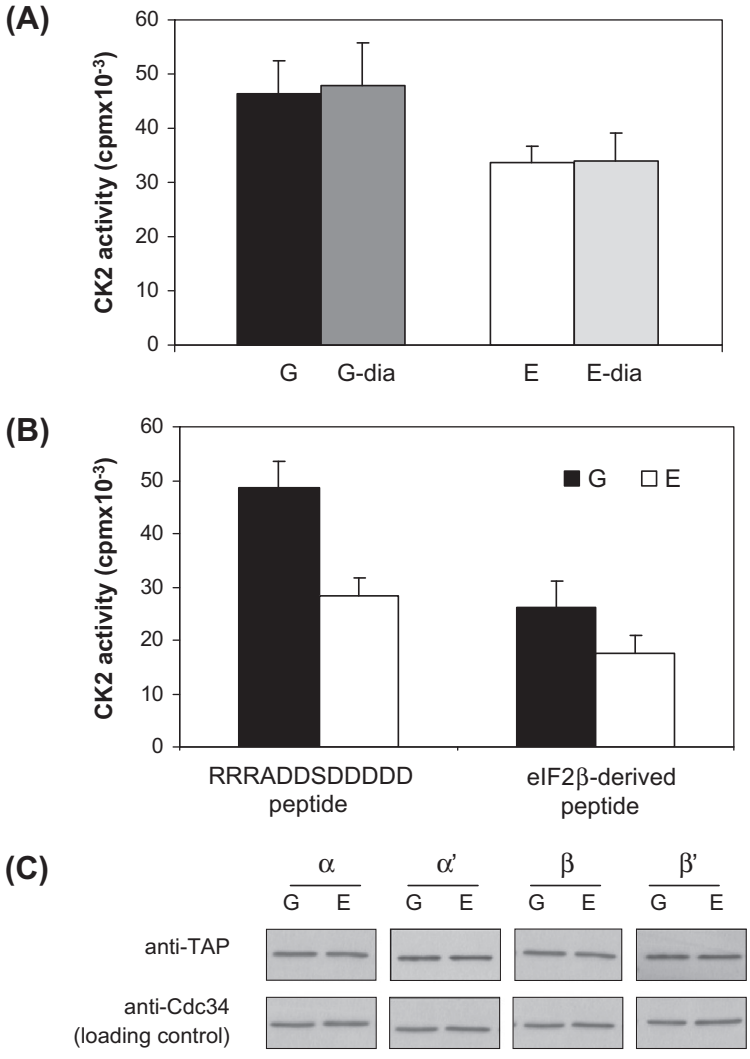


Fig. 4. CK2 activity modulation is not due to small molecules or to a different assembly of the enzyme. (A) Protein were extracted from cells growing in glucose or ethanol containing media and dialysed against fresh buffer. Protein extracts, before and after dialysis, were used to assay CK2 activity towards the peptide RRRADDSDDDDD. (B) Protein extracts from cells growing on glucose or ethanol were used to assay CK2 activity towards the peptide RRRADDSDDDDD (which can be phosphorylated both by the holoenzyme and by free catalytic subunits) and towards the eIF2β-derived peptide (which can be phosphorylated only by the holoenzyme). Means ± standard deviations are indicated ($p < 0.05$). (C) Strains expressing TAP-tagged CK2 subunits (α, α′, β, β′) were grown in medium containing glucose or ethanol as carbon source. Samples were taken in mid-exponential phase to compare the levels of CK2 subunits by western analysis using anti-TAP antibody (anti-Cdc34 antibody was used as loading control). One of three independent experiments is reported.

growing ones towards both substrates (Fig. 4B), suggesting a similar subunit composition of the enzyme in the both conditions.

The levels of the catalytic and regulatory subunits of CK2 were also investigated in cells grown in glucose and ethanol containing medium, expressing one of different TAP-tagged CK2 subunits (α, α′, β, β′). The levels of all subunits were not altered in ethanol growing cells in comparison with those of glucose growing ones (Fig. 4C).

4. Discussion

Our data provide the first evidence of *in vivo* modulation of CK2 activity in *S. cerevisiae*. Batch experiments show that CK2 specific activity is affected by nutritional conditions, being higher in conditions supporting higher growth rates (Fig. 1B and C and Fig. 2A). Let's consider cells grown in synthetic complete media supplemented with glucose and ethanol. Under our experimental conditions specific activities correspond to V_{max}, as shown by the fact that ratios for specific activities and V_{max} of cells grown in glucose vs. ethanol are 1.41 and 1.37, respectively. Since V_{max} have been normalized to the protein content and the same number of CK2 molecules/unit of protein is present in glucose- and ethanol-grown cells, changes in V_{max} can be ascribed to changes in k_{cat}. Since glucose-grown cells are larger and contain more protein than ethanol-grown cells, they also contain a larger number of CK2 molecules. Being CK2 a nuclear enzyme (Fig. 1), it is worth to remember that nuclear volume has been reported to represent a constant fraction of total cell volume, regardless of the carbon source [29]. Hence, differences in intracellular CK2 activity of glucose- and ethanol-grown cells appear to depend on both increase in molecule number and k_{cat}. The latter modulation is neither due to the presence of small-molecular activators or inhibitors, nor to a different subunit assembly of the enzyme (Fig. 4), leaving open the question of how growth rate can regulate CK2 activity; phosphorylation events or protein–protein interactions could be involved, but further studies are required to reach a better understanding of this point.

Several reports have proposed a positive correlation between CK2 activity and cellular proliferation in mammalian cells [15,18]. Mostly, these reports compared normal and transformed cells whose increased proliferation potential may reflect either an increased growth rate and/or alteration in metabolism [30]. Indeed CK2 activity – re-normalized on a per cell basis – decreases as MDT increases. Correlation with growth rate is lost at very low growth rates (*i.e.* in glycerol containing medium), suggesting that CK2 activity, which is essential for viability, cannot decrease under a certain threshold (Fig. 2B).

To dissect the contribution given to CK2 activity modulation by sensing and metabolism of the carbon source and by the growth rate, we used continuous cultures to compare cells growing at different growth rates while maintaining the same carbon source (glucose) and overall metabolism. Under glucose limitation, faster growing cells do not show alteration in K_m, but have higher V_{max} (Fig. 3B); these data are consistent with results obtained in batch cultures, where V_{max} is higher in glucose growing cells (Fig. 1C). Metabolically, cells from chemostat cultures grown at high and low dilution rates are very similar, maintaining a fully respiratory metabolism (Fig. 3). These results thus indicate that the difference in growth rate is the major factor modulating CK2 activity in yeast cells grown in glucose-limited chemostats, thus providing the first unequivocal demonstration that growth rate itself can affect CK2 activity in eukaryotic organisms.

In budding yeast many pathways are known to be nutrient-regulated, such as TOR, PKA and Snf1 pathways [31], but, to the best of our knowledge, it is not clear yet whether the modulation of the activity of these kinases is only due to nutrients as metabolites or also to their effects on growth control. To this aim, the chemostat approach employed in this study could also provide an experimental set up to distinguish between these two possibilities for other kinases.

Given the high conservation between yeast and mammalian CK2, our findings on yeast CK2 regulation could be relevant for higher eukaryotes as well.

Acknowledgments

We thank Prof. L.A. Pinna for encouraging and support and Neil Campbell for language editing. This work was partially supported by Grants to P.C. (FAR 2008), and L.A. (FIRB-ITALBIONET and UNICELLSYS).

References

[1] B. Guerra, O.G. Issinger, Protein kinase CK2 and its role in cellular proliferation, development and pathology, Electrophoresis 20 (2) (1999) 391–408.
[2] F. Meggio, L.A. Pinna, One-thousand-and-one substrates of protein kinase CK2?, FASEB J 17 (3) (2003) 349–368.
[3] D.W. Litchfield, Protein kinase CK2: structure, regulation and role in cellular decisions of life and death, Biochem. J. 369 (Pt 1) (2003) 1–15.
[4] A.C. Bibby, D.W. Litchfield, The multiple personalities of the regulatory subunit of protein kinase CK2: CK2 dependent and CK2 independent roles reveal a secret identity for CK2beta, Int. J. Biol. Sci. 1 (2) (2005) 67–79.
[5] R. Padmanabha, J.L. Chen-Wu, D.E. Hanna, C.V. Glover, Isolation, sequencing, and disruption of the yeast *CKA2* gene: casein kinase II is essential for viability in *Saccharomyces cerevisiae*, Mol. Cell Biol. 10 (8) (1990) 4089–4099.
[6] A. Rethinaswamy, M.J. Birnbaum, C.V. Glover, Temperature-sensitive mutations of the CKA1 gene reveal a role for casein kinase II in maintenance of cell polarity in *Saccharomyces cerevisiae*, J. Biol. Chem. 273 (10) (1998) 5869–5877.
[7] D.E. Hanna, A. Rethinaswamy, C.V. Glover, Casein kinase II is required for cell cycle progression during G1 and G2/M in *Saccharomyces cerevisiae*, J. Biol. Chem. 270 (43) (1995) 25905–25914.
[8] M. Faust, M. Montenarh, Subcellular localization of protein kinase CK2. A key to its function?, Cell Tissue Res 301 (3) (2000) 329–340.
[9] C.G. Penner, Z. Wang, D.W. Litchfield, Expression and localization of epitope-tagged protein kinase CK2, J. Cell Biochem. 64 (4) (1997) 525–537.
[10] O. Filhol, A. Nueda, V. Martel, D. Gerber-Scokaert, M.J. Benitez, C. Souchier, Y. Saoudi, C. Cochet, Live-cell fluorescence imaging reveals the dynamics of protein kinase CK2 individual subunits, Mol. Cell Biol. 23 (3) (2003) 975–987.
[11] R.A. Faust, G. Niehans, M. Gapany, D. Hoistad, D. Knapp, D. Cherwitz, A. Davis, G.L. Adams, K. Ahmed, Subcellular immunolocalization of protein kinase CK2 in normal and carcinoma cells, Int. J. Biochem. Cell Biol. 31 (9) (1999) 941–949.
[12] H. Wang, S. Yu, A.T. Davis, K. Ahmed, Cell cycle dependent regulation of protein kinase CK2 signaling to the nuclear matrix, J. Cell Biochem. 88 (4) (2003) 812–822.
[13] J. Sommercorn, J.A. Mulligan, F.J. Lozeman, E.G. Krebs, Activation of casein kinase II in response to insulin and to epidermal growth factor, Proc. Natl. Acad. Sci. USA 84 (24) (1987) 8834–8838.
[14] M.K. Homma, I. Wada, T. Suzuki, J. Yamaki, E.G. Krebs, Y. Homma, CK2 phosphorylation of eukaryotic translation initiation factor 5 potentiates cell cycle progression, Proc. Natl. Acad. Sci. USA 102 (43) (2005) 15688–15693.
[15] U. Münstermann, G. Fritz, G. Seitz, Y.P. Lu, H.R. Schneider, O.G. Issinger, Casein kinase II is elevated in solid human tumours and rapidly proliferating non-neoplastic tissue, Eur. J. Biochem. 189 (2) (1990) 251–257.
[16] S. Sarno, L.A. Pinna, Protein kinase CK2 as a druggable target, Mol. Biosyst. 4 (9) (2008) 889–894.
[17] J.H. Trembley, G. Wang, G. Unger, J. Slaton, K. Ahmed, Protein kinase CK2 in health and disease: CK2: a key player in cancer biology, Cell. Mol. Life Sci. 66 (11–12) (2009) 1858–1867.
[18] K. Prowald, H. Fischer, O.G. Issinger, Enhanced casein kinase II activity in human tumour cell cultures, FEBS Lett. 176 (2) (1984) 479–483.
[19] R.L. Rossi, V. Zinzalla, A. Mastriani, M. Vanoni, L. Alberghina, Subcellular localization of the cyclin dependent kinase inhibitor Sic1 is modulated by the carbon source in budding yeast, Cell Cycle 4 (12) (2005) 1798–1807.
[20] O. Marin, F. Meggio, L.A. Pinna, Design and synthesis of two new peptide substrate for the specific and sensitive monitoring of casein-kinase-1 and -2, Biochem. Biophys. Res. Commun. 198 (1994) 898–905.
[21] G. Poletto, J. Vilardell, O. Marin, M.A. Pagano, G. Cozza, S. Sarno, A. Falque's, E. Itarte, L.A. Pinna, F. Meggio, The regulatory β subunit of protein kinase CK2 contributes to the recognition of the substrate consensus sequence a study with an eIF2 β-derived peptide, Biochemistry 47 (2008) 8317–8325.
[22] P. Coccetti, R.L. Rossi, F. Sternieri, D. Porro, G.L. Russo, A. di Fonzo, F. Magni, M. Vanoni, L. Alberghina, Mutations of the CK2 phosphorylation site of Sic1 affect cell size and S-Cdk kinase activity in *Saccharomyces cerevisiae*, Mol. Microbiol. 51 (2) (2004) 447–460.
[23] D. Porro, L. Brambilla, L. Alberghina, Glucose metabolism and cell size in continuous cultures of *Saccharomyces cerevisiae*, FEMS Microbiol. Lett. 229 (2) (2003) 165–171.

[24] W.K. Huh, J.V. Falvo, L.C. Gerke, A.S. Carroll, R.W. Howson, J.S. Weissman, E.K. O'Shea, Global analysis of protein localization in budding yeast, Nature 425 (6959) (2003) 686–691.
[25] S. Sarno, H. Reddy, F. Meggio, M. Ruzzene, S.P. Davies, A. Donella-Deana, D. Shugar, L.A. Pinna, Selectivity of 4,5,6,7-tetrabromobenzotriazole, an ATP site-directed inhibitor of protein kinase CK2 ('casein kinase-2'), FEBS Lett. 496 (1) (2001) 44–48.
[26] P. Coccetti, V. Zinzalla, G. Tedeschi, G.L. Russo, S. Fantinato, O. Marin, L.A. Pinna, M. Vanoni, L. Alberghina, Sic1 is phosphorylated by CK2 on Ser201 in budding yeast cells, Biochem. Biophys. Res. Commun. 346 (3) (2006) 786–793.
[27] D. Herbert, Some principles of continuous culture, Recent Progr. Microbiol. 7 (1959) 381–396.
[28] J.P. van Dijken, J. Bauer, L. Brambilla, P. Duboc, J.M. Francois, C. Gancedo, M.L. Giuseppin, J.J. Heijnen, M. Hoare, H.C. Lange, E.A. Madden, P. Niederberger, J. Nielsen, J.L. Parrou, T. Petit, D. Porro, M. Reuss, N. van Riel, M. Rizzi, H.Y. Steensma, C.T. Verrips, J. Vindeløv, J.T. Pronk, An interlaboratory comparison of physiological and genetic properties of four *Saccharomyces cerevisiae* strains, Enzyme Microb. Technol. 26 (9–10) (2000) 706–714.
[29] P. Jorgensen, N.P. Edgington, B.L. Schneider, I. Rupes, M. Tyers, B. Futcher, The size of the nucleus increases as yeast cells grow, Mol. Biol. Cell 18 (9) (2007) 3523–3532.
[30] G. Kroemer, J. Pouyssegur, Tumor cell metabolism: cancer's Achilles' heel, Cancer Cell 13 (6) (2008) 472–482.
[31] R. Dechant, M. Peter, Nutrient signals driving cell growth, Curr. Opin. Cell Biol. 20 (6) (2008) 678–687.