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Research Article

Control of methionine biosynthesis genes by protein kinase CK2-mediated phosphorylation of Cdc34

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Abstract. Methionine and metabolites such as S-adenosylmethionine (AdoMet) are of vital importance for eukaryotes; AdoMet is the main donor of methyl groups and is involved in expression control of the methionine biosynthesis genes (MET genes). Genome-wide expression profiling of protein kinase CK2 deletion strains of the budding yeast Saccharomyces cerevisiae has indicated a function for CK2 in MET gene control. Deletion of the regulatory CK2 subunits leads to MET gene repression, presumably due to an impaired phosphorylation of the ubiquitin-conjugating enzyme Cdc34, which controls the central MET gene transcription factor Met4. We show that CK2 phosphorylates Cdc34 at two sites and one of these, Ser282, has a significant impact on MET gene expression in vivo, and that high AdoMet levels inhibit CK2. The data provide evidence for a control of MET gene expression by protein kinase CK2-mediated phosphorylation of Cdc34, and appear to suggest a feedback control loop in which high AdoMet-levels are limiting CK2 activity and thus MET gene expression.

Keywords. AdoMet, cell cycle, gene expression, methionine biosynthesis, protein kinase CK2.

Introduction

Methionine, besides representing a common constituent of proteins, is embedded into various biochemical pathways, including the ‘Methyl cycle’ which generates the key metabolite S-adenosylmethionine (AdoMet). As the main donor of methyl groups in methylation reactions, AdoMet is of vital importance. Organisms such as the budding yeast Saccharomyces cerevisiae synthesize methionine in a multi-step process, assimilating extracellular sulfate into the organic compound homocysteine, the precursor for methionine and AdoMet. The genes encoding the enzyme cascades of methionine synthesis and metabolism are known as MET genes. As well as by cell cycle [1], MET gene expression is controlled by intracellular AdoMet levels: When AdoMet concentration is low, transcription of MET genes is activated. Central to this activation is Met4, a leucine zipper family-transcription factor, which affects transcription via DNA-interacting cofactors such as Cbf1/Met28 and Met31/Met32 [2]. Repression of Met4-dependent transcription in response to high AdoMet levels is mediated by an ubiquitination complex, consisting of the ubiquitin ligase SCF^{Met30} and the ubiquitin-conjugating enzyme Cdc34, which is crucial for yeast viability [3]. Depending on cellular environment, the ubiquitination of Met4 induces proteasomal degradation or inhibition of Met4 recruitment to MET gene promoters [4]. It has been unclear how AdoMet-levels are signaled to regulate Met4 activity.

Protein kinase CK2 is a vital Ser/Thr phosphotransferase that is highly conserved in eukaryotes [5] and regarded as a survival factor [6]. It is a tetramer consisting of two

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catalytic (α) and two regulatory (β) subunits. In the budding yeast *S. cerevisiae*, two α subunit isoforms (Cka1, Cka2) complex to an obligatory tetramer with two β subunit isoforms (Ckb1, Ckb2) controlling stability, activity and substrate specificity of the holoenzyme [7]. In human cells, cell cycle entry and progression through G1 requires CK2 [5, 8–11]. Since the early cell cycle phase is critical for cell fate, regarding proliferation, differentiation or apoptosis, aberrant CK2 activity is expected to contribute to diseased states, including tumorigenesis [12–14]. To further characterize the role of CK2 at cell cycle entry, we have performed a genome-wide gene expression analysis of *S. cerevisiae*-CK2 deletion strains [15]. One of the gene groups most significantly affected by CK2 perturbation turned out to be the MET genes, showing significant repression in the regulatory CK2 subunit mutant *ckb*Δ. Recently, we have shown that Cdc34 is a substrate of CK2 [16]. Here, we present evidence that Cdc34 is phosphorylated by CK2 at two sites and that one of these, Ser282, has a significant impact on MET gene expression. The phosphorylation is inhibited by high AdoMet levels, suggesting a limiting effect on CK2 and thus involvement in AdoMet-level signaling to regulate Met4 activity.

Materials and methods

Yeast strains and growth conditions. CK2-mutant strain *ckb*Δ (YAPB10-2c, *MAT***a** *CKA1 CKA2 ckb1-Δ1::HIS3 ckb2-Δ1::LEU2*; [17]) and its wild-type (YPH499, *MAT***a** *CKA1 CKA2 CKB1 CKB2*) were generously provided by C. V. C. Glover (Biochemistry and Molecular Biology, University of Georgia, Athens, GA, USA). The diploid *CDC34*-mutant strain YC23990 (BY4743, *MAT***a**/α *YDR054c::kanMX4/YDR054C met15Δ0/MET15 leu2Δ0/leu2Δ0 lys2Δ0/LYS2 his3Δ1/his3Δ1 ura3Δ0/ura3Δ0*) was received from Euroscarf (Germany). Strains were grown in YPD medium at 30 °C, shaken at 230 rpm, if not otherwise indicated.

Synchronization. Pheromone-based synchronization was performed as described by Spellman et al. [1]. Synchrony was verified by FACS analysis according to Nash et al. [18].

RNA preparation. RNA was prepared as previously described by Ackermann et al. [19].

Quantitative RT-PCR. RT was performed using Omniscript (Qiagen). cDNA was PCR-amplified combining 2× QuantiTect SYBR Green PCR Master Mix (Qiagen), 20 pmol primers, 2 µl RT reaction product and H_2O to 20 µl. Thermal cycling was performed using the ABI Prism 7700 Sequence Detector (Perkin Elmer) with an initial activation step of 15 min at 95 °C and 45 cycles of 15 s at 94 °C, 30 s at 53 °C and 30 s at 72 °C. The following primer sequences were used, *s* and *as* indicating sense and antisense, respectively: *MET4s* (GTAGTAGCAGAGGCTTCTTT), *MET4as* (ACCGTCACTCTCTCTTGATA); *MET5s* (TAGTCA-TTCATCCTTCAAAG), *MET5as* (AATGTCCTTAG-AGAGTTGAT); *MET10s* (AGTTGAGTTTGCTACC-AATC), *MET10as* (TATTGAACAGCACAGATGAA); *MET13s* (CTACGGAATCAGAGTTCAAT), *MET13as* (GTAAGGTC-TTCTCTTCCAGA); *MET16s* (CTTAT-GGGAGAAAGATGATG), *MET16as* (CCAGTAAA-CACAGCACTTAT); *MET28s* (CAGCAGTTGTTGTA-AAAGA), *MET28as* (CTCTGTGTTCTTTCTTCTCC).

Subcloning and site-directed mutagenesis. The *CDC34* sequence of pK34-1-plasmid (a generous gift from S. Jentsch, MPI for Biochemistry, Martinsried, Germany; *see* Goebl et al. [20]) was cloned into *Hin*dIII site of the pT7-7-vector. An *Nde*I site was introduced at position –3/+3 by site-directed mutagenesis using 12.5 pmol of *s* and *as* primers, 50 ng template DNA, 1 µl dNTP-Mix (10 mM each, GIBCO), 2.5 U *Pfu* DNA-polymerase and 5 µl 10× *Pfu* buffer (Stratagene) in a total volume of 25 µl. The following PCR cycle was repeated 16 times: 30 s at 95 °C, 1 min at 55 °C, 2 min/kb template DNA at 68 °C. After *Dpn*I digestion, XL1-Blue *E. coli* were transformed with 5 µl for plasmid amplification. The final Cdc34 expression vector was created by reducing the distance between the T7-promoter and the *CDC34* start codon by about 200 bp via *Nde*I digestion and subsequent relegation, a procedure previously successfully applied in a different system [21]. For replacement of serines 207 and 282 by alanines in the protein sequence of the Cdc34 mutants, site-directed mutagenesis was carried out as before (primers available on request). For expression in yeast the *CDC34* sequence of the pK34-1 plasmid was cloned into the *Hin*dIII site of the pGAD424 vector (Clontech) thereby replacing the GAL4-AD-cDNA. Two *Sal*I sites were introduced and analogously to the pT7-7-Cdc34 expression vector, the distance between promoter and *CDC34* startcodon was reduced by *Sal*I digestion and subsequent religation. Cdc34 mutant expressing vectors were created by site-directed mutagenesis as before. All subcloning and mutagenesis steps were verified by sequencing.

Recombinant proteins. Protein expression in *Escherichia coli* and purification was performed essentially according to [22]. *E. coli* BL21-cells were transformed with expression vectors carrying wild-type or mutated versions of Cdc34. Cultures were grown to $OD_{600} \sim 0.5$ at 37 °C and 250 rpm. IPTG was added to 1 mM, followed by 3 h incubation. Cells were harvested, and the pellet was resuspended in extraction buffer (50 mM Tris-HCl pH 7.4, 2 mM EDTA, 0.5 mM EGTA, 1 mM DTT, 2% glycerol, 1% Triton X-100) followed by sonification.

After DNase-I digestion (2 µg/ml, 30 min, 4 °C) extracts were centrifuged two times (20 min, 3000 *g*/90 min, 20 000 *g*), and supernatant was applied to a DEAE-Sepharose column (Pharmacia), washed with 5 vol buffer A (50 mM Tris-HCl pH 7.4, 2 mM EDTA, 0.5 mM EGTA, 1 mM DTT, 2% glycerol, 0.1 M NaCl) followed by elution with 0.3 M NaCl in buffer A. After applying to a Poros™ HQ column (Boehringer), washing with 5 vol buffer B (50 mM Tris-HCl pH 7.4, 10 mM $MgCl_2$, 1 mM DTT, 2 mM EDTA, 0.5 mM EGTA, 2% glycerol, 0.3 M NaCl) and elution with a linear gradient (0.3–1 M NaCl) of buffer B, collected fractions were separated by SDS-PAGE and checked for Cdc34 presence and purity. Combined positive fractions were concentrated in storage buffer (50 mM Tris-HCl pH 7.4, 2 mM EDTA, 0.5 mM EGTA, 1 mM DTT, 15% glycerol, 1 M NaCl) with YM-10 filters (Centricon), and preparations stored at –80 °C.

Preparation of yeast nuclear extracts. Yeast nuclear extracts were prepared as described by Ausubel et al. [23].

Phosphorylation assay. Recombinant yeast Cdc34 protein (20 pmol) were incubated with 1 pmol recombinant CK2 subunits [24] or 20 µg total protein from yeast nuclear extract, respectively, in 25 µl kinase reaction buffer (50 mM Tris-HCl pH 7.5, 12 mM $MgCl_2$, 100 mM NaCl, 40 µM ATP, 10 µCi [γ-^{32}P]ATP) at 37 °C for 40 min; 5 µl of the reaction mix were separated by SDS-PAGE. After Coomassie blue staining, the gel was dried on a vacuum dryer and a phosphoimage was prepared by exposure at room temperature for 12 h. Scanning was performed with a BAS-1500 IP-Reader (Fujifilm) and signals were quantified by TINA 2.09 software. CK2 activity in presence of different AdoMet concentrations was assayed with $\alpha_2\beta_2$-CK2 holoenzyme (15 nM) and a model peptide substrate RRRAADSDDDDD (0.2 mM). Aliquots of the reaction mix, 20 µl, were pipetted on P81 Whatman paper stripes that, after air drying, were washed in 0.5% H_3PO_4 (2 × 10 min) and shortly immersed in acetone. After air drying, radioactivity was determined in a scintillation counter.

Transformation of yeast cells. Yeast cells were transformed following the LiAc-protocol [23].

Sporulation. Sporulation induction and colony selection of yeast strain YC23990 transformed with pGAD-Cdc34 expression vectors was performed as described by Ausubel et al. [23]. Haploid genotypes were confirmed by replica plating.

Results

CK2 perturbation affects MET gene expression. In genome-wide expression screens of *S. cerevisiae*-CK2 wild-type and deletion strains following release from a G0-like (pheromone-arrested) state, absence of regulatory CK2 subunits (strain *ckb*Δ) caused significant repression of several MET genes [15]. Previously, these genes had been identified by Spellman et al. [1] to be cell cycle-regulated as a part of the so-called 'MET cluster'. The CK2 perturbation effect, however, appears not to be a cell cycle entry-specific feature, since MET gene repression can also be detected in asynchronous *ckb*Δ cultures [19].
To verify the array data, we performed a quantitative RT-PCR analysis. This confirmed that MET genes, compared with wild-type, are significantly repressed in *ckb*Δ strains before and during entry into cell cycle, while expression of the central MET gene transcription factor Met4 is not altered (Fig. 1a). MET genes that were not regulated by cell cycle progression were also included in our PCR analysis, but we noticed that virtually the whole methionine biosynthesis pathway relates to CK2-dependent expression, from sulfate uptake to the final methionine product: ATP sulfurylase gene *MET3*, adenylsulfate kinase gene *MET14*, phosphoadenylsulfate reductase gene *MET16*, sulfite reductase genes *MET5* and *MET10*, homoserine transacetylase gene *MET2*, *O*-acetylhomoserine-*O*-acetylserine sulfhydrolase gene *MET25*, and homocysteine methyltransferase gene *MET6* (Fig. 1b).

Phosphorylation of Cdc34 by recombinant CK2. Since CK2 perturbation significantly affects the expression of MET genes but not the expression of their central element of regulation, the transcription factor Met4, the question was how the CK2 effect may be mediated. Met4 is known to be controlled post-translationally by the Cdc34-SCF^{Met30} ubiquitination complex, but ubiquitination of Met4 is not induced by its phosphorylation [25], as is the case with most SCF substrates [26]. Thus, other regulatory mechanisms must exist. Interestingly, examining the Wnt signaling pathway, Semplici et al. [27] noted that CK2-mediated phosphorylation of the human Cdc34 homologues UBC3 and UBC3B enhances their interaction with the F-box protein β-TrCP, causing increased β-catenin degradation. Moreover, yeast Cdc34 has previously been identified by *in silico* analysis as a high-probability CK2 substrate [7]. The proposed phosphorylation site was Ser282 in its C-terminal tail, which is supposed to play a role for substrate specificity [28]. A sequence check indicates that in addition to Ser282, Cdc34 contains another potential CK2-motif, Ser207, localized in an essential domain (amino acids 171–209) crucial for its cell cycle function [29].
Recently, we have shown that Cdc34 is in fact a CK2 substrate. Performing phosphorylation assays with recombinant Cdc34 and CK2 subunits, we found that Cdc34 is strongly phosphorylated by CK2 holoenzyme, and that phosphorylation is significantly decreased when the regulatory subunits are removed [16]. This result was obtained

similarly with holoenzymes containing α, α', or both as the catalytic part (data not shown). To determine the sites phosphorylated by CK2, we created Cdc34 mutants carrying Ser-to-Ala substitutions at positions Ser282 and/or Ser207, respectively. Compared to the phosphorylation of wild-type Cdc34, the phosphorylation efficiency of the *S282A* mutant was reduced down to about 20%, and an even stronger reduction was observed for the *S207A* mutant (Fig. 2a). As with the wild-type protein, phosphorylation of both Cdc34 mutant forms was strongly enhanced by the presence of CK2β. Replacement of both CK2 sites in Cdc34, mutant *S207/282A*, resulted in

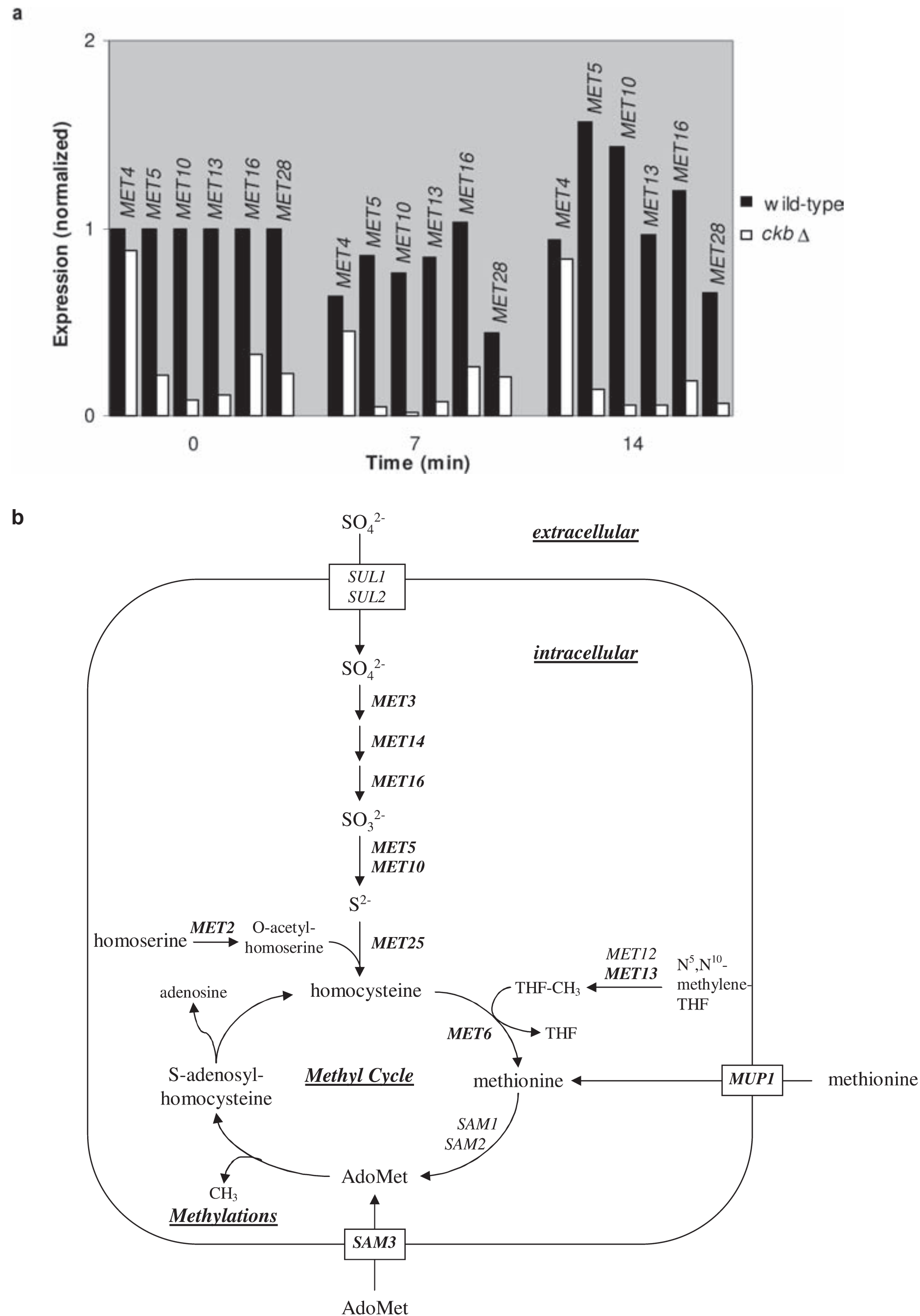

Figure 1. Effects of CK2 perturbation on the expression of MET genes. (*a*) MET gene repression in regulatory CK2 subunit deletion strain *ckb*Δ was determined by quantitative RT-PCR. Representative MET gene transcript levels at 0, 7 and 14 min after release from pheromone-induced G0/G1 arrest are given. As a reference, wild-type transcript levels at 0 min were set to 1 for each gene. Shown are the mean values of at least three independent experiments (standard deviations below 5%). (*b*) Methionine biosynthesis pathway in yeast. Gene names for enzymes catalyzing single biosynthetic steps are given; those printed in bold show CK2-dependent expression. For further information see text. *THF*, tetrahydrofolate; *AdoMet*, S-adenosylmethionine.

a further decrease in phosphorylation (phosphorylation undetectable with CK2α alone). The autophosphorylation of CK2β was, as expected, comparably high in all experiments carried out with CK2 holoenzyme, providing a positive internal control of assay.

CK2-specific phosphorylation of Cdc34 by nuclear extracts. Cdc34 is primarily found in the nucleus [30] where also a main quantity of CK2 is located [7]. To get further evidence whether Cdc34 might be phosphorylated by CK2 *in vivo*, we performed phosphorylation assays with Cdc34 and yeast nuclear extracts. As a result, Cdc34 was significantly phosphorylated by CK2 wild-type extracts (Fig. 2b), and phosphorylation was dramatically reduced in the presence of TBB (tetrabromobenzotriazol), a specific inhibitor of CK2 [31]. Compared to wild-type, extracts of *ckb*Δ cultures exhibited a significantly lower Cdc34 phosphorylation activity, indicating again an important role of the regulatory CK2 subunits. For both, the *ckb*Δ strain and the wild-type extracts, mutation of CK2 site Ser282 or Ser207 in Cdc34 led to a significant decrease in phosphorylation, while double-mutant Cdc34-*S207/282A* did not show any phosphorylation signal at all (Fig. 2b).
From these results, we concluded that Cdc34 is phosphorylated by protein kinase CK2 at sites Ser282 and Ser207, and that these positions are the only relevant CK2 phosphorylation sites in the Cdc34 protein.

AdoMet-mediated inhibition of CK2 activity. Met4 activity and thereby expression of MET genes, is dependent on intracellular AdoMet levels. Assuming a role for CK2-mediated phosphorylation of Cdc34 in MET gene expression, we were interested in assessing whether CK2 was affected by AdoMet. Indeed, performing phosphorylation assays with CK2 at different AdoMet concentrations, we observed a dose-dependent inhibition of CK2 activity. When determined at levels of 15 nM CK2 and 0.2 mM peptide substrate, a 50% inhibition was reached at a 2–5 mM AdoMet level (Fig. 3a), and inhibition was increased upon increasing the AdoMet level.
Not only was the phosphorylation of a CK2 peptide substrate inhibited, the presence of AdoMet also resulted in a significantly reduced phosphorylation of Cdc34 by nuclear extracts of strains of both CK2 wild-type and *ckb*Δ (Fig. 3b). This AdoMet-mediated inhibition further supported the idea of a role for CK2 in MET gene regulation via phosphorylation of Cdc34.

Deletion of CK2 phosphorylation sites in Cdc34 affects MET gene expression *in vivo*. It has been shown previously that Cdc34 exists as a phosphoprotein *in vivo* [30]. However, neither the phosphorylation sites, nor the responsible kinase, nor the function of phosphorylation have been identified so far. To investigate a potential role of CK2-mediated Cdc34 phosphorylation for MET gene

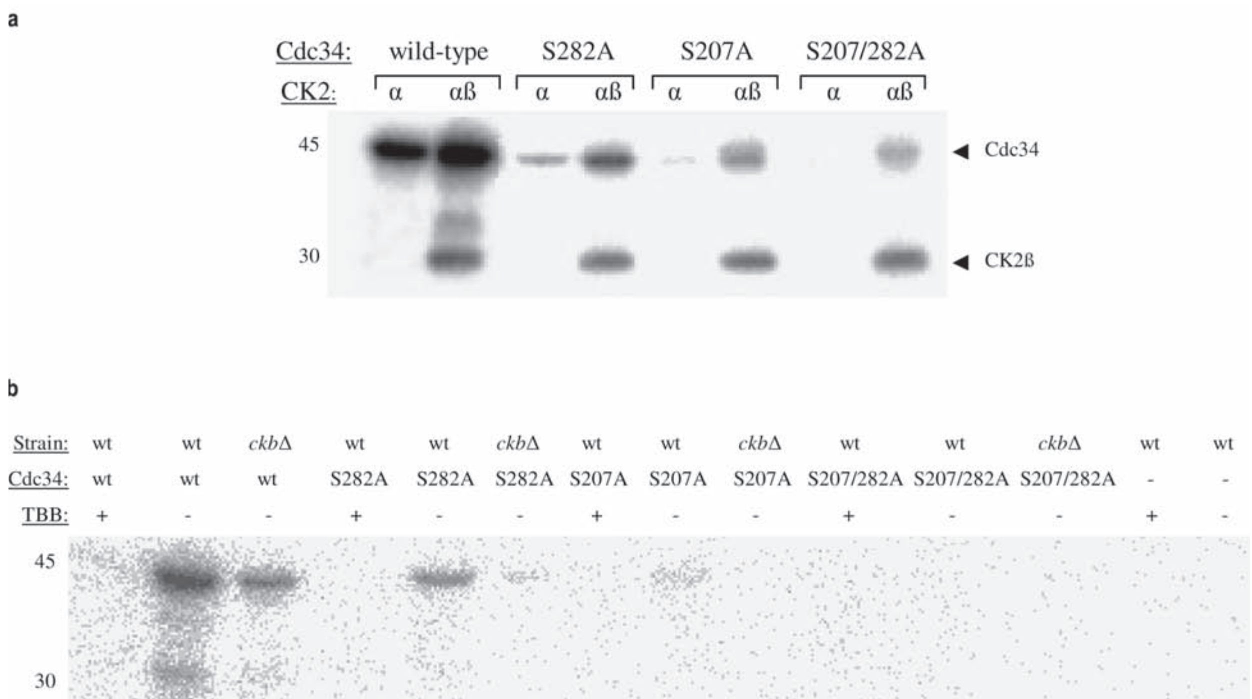

Figure 2. CK2-mediated phosphorylation of ubiquitin-conjugating enzyme Cdc34. (*a*) Phosphorylation of Cdc34 wild-type and CK2-site mutants by catalytic CK2 subunit α and CK2 holoenzyme. Shown is an autoradiography image of SDS-PAGE-separated phosphorylation assay reactions with the indicated Cdc34 und CK2 proteins. Protein sizes are given in kDa. (*b*) CK2-specific phosphorylation of Cdc34 by yeast nuclear extracts of *ckb*Δ compared with wild-type. CK2-specific inhibitor TBB (tetrabromobenzotriazol) was added at a concentration of 60 µM where indicated.

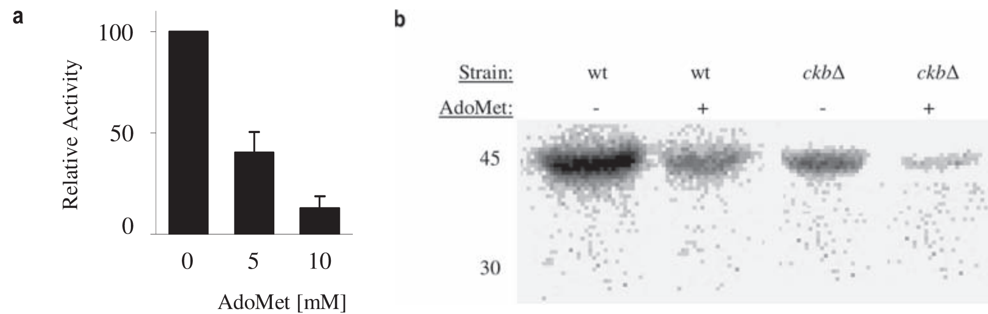

Figure 3. AdoMet-mediated inhibition of CK2 activity. (*a*) Dose-dependent inhibition of CK2 by AdoMet. Phosphorylation assays carried out with $\alpha_2\beta_2$-CK2 holoenzyme (15 nM) and model peptide substrate RRRAADSDDDDD (0.2 mM). Phosphorylation inhibition at the given AdoMet concentrations were determined under linear conditions of phosphorylation kinetics and CK2 concentration (deviations below 5%). Error bars indicate standard deviations of four independent experiments. (*b*) CK2-specific phosphorylation of Cdc34 by yeast nuclear extract and its inhibition by AdoMet. Assay carried out with nuclear extracts from wild-type and *ckb*Δ strains in presence of AdoMet at a concentration of 5 mM where indicated. Shown is an autoradiographic image of SDS-PAGE-separated phosphorylation assay reactions; protein sizes are given in kDa.

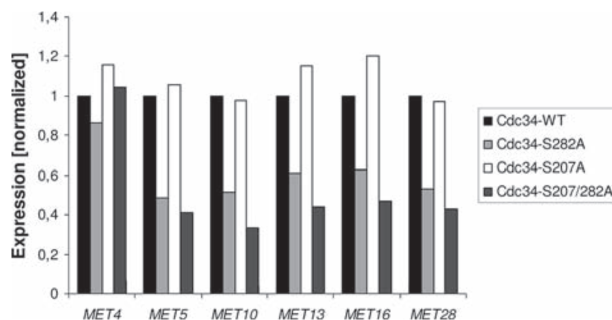

Figure 4. *In vivo* effect of deleted CK2 phosphorylation sites in Cdc34 on MET gene expression. Transcript levels of representative MET genes in *cdc34*-knockout cells with extrachromosomal expression of Cdc34 wild-type protein (Cdc34-WT) or of one of the Cdc34 CK2-site mutant forms (Cdc34-*S282A*; Cdc34-*S207A*; Cdc34-*S207/S282A*) are shown. Wild-type expression of each MET gene was set to 1. Shown are the mean values of at least three independent experiments (standard deviations below 5%).

expression, we replaced native Cdc34 by different Cdc34 mutant forms *in vivo*. Haploid colonies were generated from a diploid, heterozygous *CDC34* strain, transformed with Cdc34 expression vectors. Clones carrying the *cdc34*Δ allele and extra-chromosomally expressing either Cdc34 wild-type or one of the Cdc34 mutant forms for the CK2 phosphorylation sites were investigated for MET gene transcription. Compared to wild-type, no expression deviations were detected in the Cdc34-*S207A* mutant culture. However, MET genes were significantly repressed in the Cdc34-*S282A* as well as in the Cdc34-*S207/282A* double-mutant cultures, while the transcript levels of Met4 were, as expected, unaltered in all mutants (Fig. 4).

From these *in vivo* data, we conclude that protein kinase CK2 has a positive impact on the expression of MET genes via phosphorylation of Cdc34 at site Ser282. Together with the inhibition of CK2 by higher levels of AdoMet as described above, this provides a possible explanation of how AdoMet levels could be signaled to regulate Met4 activity and thus MET gene transcription.

Discussion

The biosynthesis of sulfur containing amino acids is highly conserved in eukaryotes [32]. By the description of *met* mutants arresting in G1 under methionine depletion, and methionine-tRNA-synthase mutants arresting in G1 despite the presence of methionine, Unger and Hartwell [33] for the first time presented connections between methionine metabolism and cell cycle control in *S. cerevisiae*. Similar links have also been found in mammals: *in vitro* as well as *in vivo* studies show that certain tumor cells are specifically dependent on methionine and – in contrast to normal, untransformed cells – are not able to use homocysteine instead [34, 35]. Another indication for a link between cell cycle control and methionine biosynthesis in yeast has been presented by Spellman et al. [1], showing cell cycle-regulated expression of 20 MET cluster genes peaking in S-phase. Here we demonstrate that MET gene transcription in yeast is significantly repressed, when the function of protein kinase CK2 is perturbated by the absence of its regulatory subunits.

MET gene expression is activated by two heteromeric complexes both including the central MET gene transcription factor Met4. Activity of Met4 is regulated by cooperated action of the ubiquitin-conjugating enzyme Cdc34 and the SCF^{Met30}-ubiquitin-ligase in an AdoMet-dependent manner. However, Met4 does not show any phosphoforms relating to AdoMet levels [25], so that it has been unclear, how Met4 inactivation is induced when sufficient methionine has been synthesized. CK2-mediated phosphorylation of the human Cdc34 homologues UBC3 and UBC3B enhances ubiquitin-dependent proteolysis of β-catenin in the Wnt pathway [27] and affects cellular localization, which is probably based on a specific

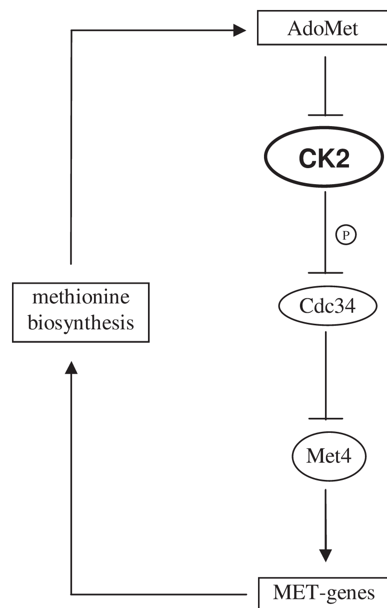

Figure 5. Hypothetic model of MET gene regulation by CK2 via AdoMet-controlled phosphorylation of Cdc34. For details see text.

interaction between Cdc34 and the CK2β subunit [36]. Consistent with that, we show that CK2 also phosphorylates yeast Cdc34 at positions Ser207 and Ser282, with a strong enhancing effect by the regulatory CK2β subunits. In addition, Cdc34 is phosphorylated at the same sites by yeast nuclear extracts in a CK2-specific manner, as indicated by a strikingly reduced phosphorylation in the presence of CK2-inhibitor TBB. Moreover, the positive effect of the regulatory CK2 subunits is confirmed by a significantly decreased phosphorylation of Cdc34 by the *ckb*Δ mutant compared with wild-type.

Consistent with our results, Goebl et al. [30] have identified Cdc34-phosphoforms exclusively with phosphoserines *in vivo*. In further agreement, the phosphosites they detected are located in the main part of the Cdc34 protein as well as within its 42 C-terminal amino acids where – beside the CK2-motif Ser282 – only one other potential phosphorylation site (for protein kinase C) is localized. While until now, we have not determined a physiological function for the phosphorylation at Ser207, we demonstrate here that a *S282A* mutation leads to significant MET gene repression *in vivo*. This inhibitory effect of CK2-mediated phosphorylation on Cdc34 function, resulting in a decreased Met4 inactivation, appears to be different from the human situation, where the phosphorylation by CK2 enhances Cdc34 action on β-catenin [27]. However, since these observations were made in different contexts (MET pathway – Wnt pathway), this might be due to different Cdc34 regulatory properties depending on specific substrates and/or interaction partners. In addition, we show here that CK2 activity is inhibited by AdoMet at higher levels, a key metabolite of the methionine biosynthesis pathway. Our results seem to suggest the possibility of a closed regulation loop in MET gene expression (Fig. 5). Accordingly, protein kinase CK2 would contribute to the control of MET gene expression in an AdoMet level-dependent manner by inhibitory phosphorylation of Cdc34 at Ser282. Thus, low AdoMet levels would allow a high phosphorylation state of Cdc34, leading to high Met4 activity and, consequently, MET gene transcription. The so increased concentration of methionine biosynthesis enzymes would increase the level of AdoMet which, having reached a critical level, inhibit CK2-mediated phosphorylation of Cdc34 and thus Met4-mediated expression of MET genes, eventually establishing a negative feedback loop. AdoMet levels far above normal may also result from particular conditions, such as extracellular methionine supplementation [37].

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