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A global view of CK2 function and regulation

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Abstract

The wealth of biochemical, molecular, genetic, genomic, and bioinformatic resources available in *S. cerevisiae* make it an excellent system to explore the global role of CK2 in a model organism. Traditional biochemical and genetic studies have revealed that CK2 is required for cell viability, cell cycle progression, cell polarity, ion homeostasis, and other functions, and have identified a number of potential physiological substrates of the enzyme. Data mining of available bioinformatic resources indicates that (1) there are likely to be hundreds of CK2 targets in this organism, (2) the majority of predicted CK2 substrates are involved in various aspects of global gene expression, (3) CK2 is present in several nuclear protein complexes predicted to have a role in chromatin structure and remodeling, transcription, or RNA metabolism, and (4) CK2 is localized predominantly in the nucleus. These bioinformatic results suggest that the observed phenotypic consequences of CK2 depletion may lie downstream of primary defects in chromatin organization and/or global gene expression. Further progress in defining the physiological role of CK2 will almost certainly require a better understanding of the mechanism of regulation of the enzyme. Beginning with the crystal structure of the human CK2 holoenzyme, we present a molecular model of filamentous CK2 that is consistent with earlier proposals that filamentous CK2 represents an inactive form of the enzyme. The potential role of filamentous CK2 in regulation *in vivo* is discussed. (Mol Cell Biochem **274**: 163–170, 2005)

Key words: bioinformatics, filamentous CK2, genomics and proteomics, molecular modeling, protein kinase CK2

Introduction

Protein kinase CK2 is traditionally described as an acidic-directed, constitutively active Ser/Thr protein kinase of broad substrate specificity that is ubiquitous in eukaryotic organisms (for review see [1, 2]). The enzyme is typically composed of catalytic α and regulatory β subunits that combine to form a native $\alpha_2\beta_2$ holoenzyme. Higher-order aggregates of the holoenzyme occur under physiological conditions *in vitro*, but the existence and relevance of these forms *in vivo* remain to be established [3, 4]. The large number of substrates and the absence of a known mechanism of regulation continue to be major obstacles to determining the physiological role of this enzyme.

S. cerevisiae CK2 is composed of two catalytic subunits, α and α', and two putative regulatory subunits, β' and β', which are encoded by the *CKA1*, *CKA2*, *CKB1*, and *CKB2* genes, respectively (for review see [5]). Analysis of null and conditional alleles of these genes has defined roles for the enzyme in cell viability [6], cell cycle progression [7], cell polarity [8], ion homeostasis [9], recovery from double-strand break arrest [10], and other functions, while extragenic suppression studies have identified potentially interacting functions, including the pleiotropic suppressor Zds1,2 [11] and the Hsp90

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cochaperone, Cdc37 [12]. Combined biochemical and molecular/genetic studies have identified a number of probable physiological substrates, including topoisomerase II [13], eIF-2α [14], TATA-box binding protein [15], and Cdc37 [12], among others. Mutation of the CK2 phosphorylation sites of Cdc37 results in severe growth and morphological defects, indicating an important role for this phosphorylation event. Subsequent analysis has uncovered a positive feedback loop between CK2 and Cdc37 that is required for the activity of multiple protein kinases [12]. This system is conserved in mammals, suggesting an ancient relationship [16]. While phosphorylation of this one target is almost sufficient to explain the essential requirement for CK2, bioinformatic analysis suggests that there are likely to be hundreds of CK2 targets in yeast [5]. Defining the global role of the enzyme is thus a daunting task.

The growing body of genomic, proteomic, and functional genomic data available in *S. cerevisiae* [17] makes this organism an excellent system for a renewed effort to define the *in vivo* function of this enigmatic kinase. Here, we survey genomic and proteomic data available on CK2 in *S. cerevisiae* in an effort to define the physiological role of the enzyme. Collectively, the results suggest a global role for CK2 in chromatin structure and/or gene expression. Based on the recent X-ray structure of human CK2, we present a detailed molecular model of filamentous CK2 and suggest that this form of the enzyme is inactive. A consideration of the available data suggests that CK2 may serve as its own negative regulator and further that the enzyme may function as a sensor of intracellular ionic conditions.

Materials and methods

Bioinformatic analyses

Web-based searches for potential CK2 substrates in *S. cerevisiae* were performed using PROSITE (http://us.expasy.org/prosite/) [18] and ScanSite (http://scansite.mit.edu/) [19]. Other bioinformatic searches were performed using the web interface of the Saccharomyces Genome Database (http://www.yeastgenome.org/).

Molecular modeling of CK2

SWISS-MODEL (http://swissmodel.expasy.org/) and Swiss-PdbViewer [20] were used to construct a symmetric model of human CK2, to construct corresponding homology models of *D. melanogaster* and *S. cerevisiae* CK2, and to construct models of filamentous CK2 from each of these holoenzymes.

To construct a symmetric model of human CK2, two copies of the X-ray crystal structure of the human CK2 holoenzyme (PDB 1JWH) were loaded into Swiss-PdbViewer, and the Fit Selected Residues command was used to superimpose residues 7-205 of chain C of one copy onto the corresponding residues of chain D of the other (backbone atoms only were fit). Chains B and D were then deleted from each tetramer, and the Merge Layers command was used to merge the remaining subunits into a single pdb file. This file contains an approximately symmetric ACCA tetramer composed of two copies of chain A (catalytic subunit, plus bound AMPPNP) and two of chain C (regulatory subunit, plus bound zinc ion).

To construct homology models of the *D. melanogaster* and *S. cerevisiae* CK2 holoenzymes, Swiss-PdbViewer was used to save the A and C subunits of 1JWH as separate pdb files, and the appropriate A or C structure was opened in Swiss-PdbViewer to serve as the modeling target. A FASTA file of the desired Drosophila or yeast sequence was then imported using the Load Raw Sequence to Model function, and the two sequences were aligned manually in the Alignment window. The resulting sequence and structural alignment were saved as a project and submitted in Project mode to the SWISS-MODEL server for optimization. Homology models were successfully built for the α and β subunits of *D. melanogaster* CK2 and the α, α', β, and β' subunits of *S. cerevisiae* CK2. The Fit Selected Residues command of Swiss-PdbViewer was then used to superimpose each Drosophila or yeast subunit onto the corresponding subunit(s) of the symmetric human tetramer to generate the corresponding Drosophila or yeast holoenzyme. The sequence of subunits in the yeast holoenzyme was assumed to be α–β–β'–α' [5].

To construct a model of filamentous CK2, the human CK2 symmetric tetramer was opened in Swiss-PdbViewer, and the Add Residue command was used to add the missing residues Ser2–Glu5 onto the N-terminal arm of each copy of the β subunit. Residues Ser2–Ser8 of each β subunit were converted to a beta sheet conformation and identically extended away from the body of the holoenzyme by altering the phi–psi angles of Val7–Ser8 within allowed regions of the Ramachandran plot. Two of these modified symmetric tetramers were then manually docked (while viewing in stereo) to obtain a dimer of tetramers in which Ser2 of a β subunit from one tetramer is juxtaposed to the gamma phosphoryl group of an ATP (AMPPNP) of the other tetramer, and *vice versa*. The four chains in one tetramer were renamed to make them distinct from those in the other, the two layers were merged, and this dimer of tetramers was saved as a new file. The identical docking geometry was then propagated to generate a uniform helical filament. To accomplish the latter, two copies of the dimer of tetramers were opened, and specific subunits were superimposed to yield a trimer of tetramers in which the middle tetramer was present twice. One of the middle tetramers was deleted, and the resulting trimer of tetramers was merged

and save as a new file. This process was reiterated twice more to generate a filament of five tetramers. A similar approach was used to construct filamentous Drosophila and yeast CK2.

Results

Bioinformatic analysis of CK2 function in S. cerevisiae

A previous computer-based search of the *S. cerevisiae* proteome, carried out when the genome was approximately 80% complete, identified over 100 proteins containing one or more strong matches to the CK2 consensus sequence [5]. These proteins were strongly biased toward proteins involved in chromatin structure and DNA replication; RNA synthesis, transport, and turnover; protein synthesis, targeting, and degradation; cell cycle progression and cell polarity; and ion homeostasis. By contrast, few proteins involved in metabolism were identified. Subsequent Prosite searches of the completed genome yielded similar results, as did analogous searches carried out with the Scansite algorithm using the default CK2 matrix (data not shown). A number of predicted substrates have been confirmed by biochemical and/or genetic studies (e.g., see "Introduction"), and the *in vivo* phosphorylation of other predicted sites has been demonstrated by global analysis of the yeast phosphoproteome [21].

Several studies have attempted to define the pattern of global protein–protein interactions in *S. cerevisiae* [22–24], and interactions involving CK2 have been detected in several of these. In the most informative study, one or more CK2 subunits were detected in 15 of 589 tandem affinity purifications of chromosomally tagged open reading frames expressed from the endogenous promoter and in 11 of 232 multiprotein complexes deduced from these primary data [23]. Four of these complexes (70, 113, 137, and 156) contained all four subunits of CK2. Three of these complexes are categorized as having a role in transcription/DNA maintenance/chromatin structure and the fourth in RNA metabolism. Complex 70 includes several chromatin remodeling enzymes (Chd1, Isw1, Isw2), complex 113 includes a large number of RNA polymerase II elongation factors, including the core subunits of the FACT transcriptional elongation complex (Spt16 and Pob3) [25], and complex 156 contains numerous components of the U3 snoRNP. The presence of CK2 in such complexes suggests a global role for CK2 in chromatin structure and/or gene expression. The presence of known or predicted substrates of CK2 (e.g., Chd1, Spt16) in these complexes suggests that recruitment of CK2 to these complexes may promote phosphorylation of specific chromatin-associated targets. The absence of CK2 from the several defined complexes directly involved in cell cycle progression, cell polarity, etc. suggests that the observed phenotypic effects of CK2 depletion may be secondary to global effects on gene expression.

Transcriptional profiling of CK2 deletion mutants has been carried out by two groups. Both *cka2* and *ckb2* were included in the compendium of deletion mutants analyzed by Hughes *et al*. [26], who reported that both mutants yield expression changes in a set of stress response and other genes that also exhibit intrinsic variation in wild-type cells. The *cka2* mutant was also analyzed in a subsequent screen for genes required for processing of noncoding RNAs, but no statistically meaningful effect was observed [27]. In a more focused study, Barz *et al*. [28] analyzed global gene expression in synchronized cultures of *cka1*, *cka2*, and *ckb1 ckb2* null strains at defined points in the cell cycle. A significant fraction of known cell cycle genes exhibited altered expression at different points in the cell cycle, as did numerous genes involved in chromatin modification and remodeling. Alterations in global chromatin architecture elicited by the latter changes may in part underlie the observed changes in cell cycle and other gene expression. The null strains analyzed in both of these studies have little phenotypic deficit in rich medium. Use of conditions that elicit a stronger phenotype (e.g., high salt) or strains with a greater CK2 deficit is expected to yield more dramatic changes in expression [26].

Synthetic Genetic Array (SGA) analysis allows high throughput screens for synthetic genetic interactions between two non-lethal mutants in *S. cerevisiae* [29]. Although no CK2 subunit gene has yet been used as a bait in such a screen, three of the four subunits have been identified as prey in screens using other baits. A synthetic slow-growth phenotype is seen between *cdc7* and each of *cka1*, *ckb1*, and *ckb2*; between *myo2* and both *ckb1* and *ckb2*, and between *mns1* and *ckb1* only. *CDC7* encodes an essential serine/threonine protein kinase that is required throughout S phase for replication origin firing. Although CK2 is required for cell cycle progression in G1 and G2/M in *S. cerevisiae*, the enzyme is not required for S phase [7]. Interestingly, Cdc7 is the closest homolog of Cka1 and 2 in *S. cerevisiae*, raising the possibility that crosstalk between the two enzymes may explain their synthetic interaction. *MYO2* encodes a type V muscle myosin required for actin-mediated delivery of vesicles to sites of polarized growth, and may be relevant to the cell polarity defects observed in CK2 catalytic subunit mutants at nonpermissive temperature [6, 8]. Both *cdc7* and *myo2* exhibit a large number of synthetic interactions (45 each), suggesting that their interactions with CK2 may be relatively nonspecific. *MNS1* encodes an α-mannosidase activity. The interaction between *msn1* and *ckb1* may be relevant to the flocculation phenotype of CK2 mutants [6]. Full characterization of the genetic interactions displayed by CK2 awaits the use of CK2 subunits as bait in such experiments.

Global *in vivo* localization of yeast proteins has been achieved by systematic tagging of each open reading frame with green fluorescence protein (GFP) [30]. Each of the four yeast CK2 subunit genes has been successfully analyzed by

this technique. The *in vivo* localization is the same in all four cases: strong nuclear staining coupled with faint cytoplasmic staining that is clearly above background. The localization of yeast CK2, at least in logarithmic cultures growing in rich media, is thus identical to that reported earlier by Nigg's group for CK2 of vertebrate cells: in both cases CK2 is "a predominantly nuclear enzyme" [31].

The transcriptional profiling response of the four CK2 subunit genes to various physiological, genetic, or environmental conditions is generally flat, indicative of a housekeeping function whose expression is constitutive. The steady state abundance of each transcript has been estimated from high-density oligonucleotide arrays at approximately 2 copies per cell, about average for a yeast transcript [32]. The codon adaptation index of the four open reading frames is 0.178, 0.164, 0.126, and 0.150, respectively, implying a moderate and nearly stoichiometric expression level; and direct measurement of the expression level of tagged fusion constructs indicates that the α, α', β, and β' subunits are present at 7000, 4500, 8000, and 7000 molecules per cell, respectively, slightly above the median expression level of yeast proteins [33]. All of these data are consistent with a moderate, constitutive expression of the enzyme, suggesting that any regulation of enzyme activity occurs post-translationally.

Molecular modeling of filamentous CK2

The absence of a known mechanism of regulation is perhaps the greatest obstacle to defining the physiological role of CK2. The putative regulatory β subunit stimulates the activity of the catalytic subunit against most substrates but inhibits it against others (e.g., calmodulin), mediates the stimulatory effects of both polyamines and polycations, and undergoes autophosphorylation, but the physiological importance of these effects remains to be established [2, 34]. Phosphorylation of both subunits of CK2 by other kinases has been described in mammalian systems, but again the physiological relevance of these modifications has not been defined [2]. Additional proposals are that CK2 activity is regulated *via* holoenzyme assembly, intracellular translocation, or substrate availability [2, 35], or by reversible polymerization of the holoenzyme into linear filaments or other aggregates [3, 4]. How and indeed whether CK2 is regulated *in vivo* remains an open question.

The recent structure of the human CK2 holoenzyme [36] has revived interest in the early proposal that filamentous CK2 represents an inactive form of the enzyme and hence that reversible polymerization may regulate enzyme activity [3]. Figure 1 presents a diagram of this model, together with the known mechanism of regulation of cyclic AMP-dependent protein kinase (cAMPdPK), which served as part of its initial inspiration. This hand-drawn figure, which dates from 1986, was intended for inclusion in the manuscript reporting the discovery of filamentous CK2 [3], but was removed at the request of the reviewer, who thought a figure premature in the absence of data that filaments are inactive and exist *in vivo*. Here we present molecular modeling data that support the contention that filamentous CK2 is inactive against exogenous substrates but potentially capable of autophosphorylation. Biochemical data consistent with this model have recently been obtained by Meggio [37] (see "Discussion").

In the crystal structure of the $\alpha_2\beta_2$ holoenzyme of human CK2, the autophosphorylation sites on the β subunits are more than 40 Å away from the catalytic sites of the α subunits [36]. Barring a gross conformational rearrangement of the subunits, autophosphorylation within the context of a single tetramer (*cis*-autophosphorylation) thus appears impossible, strongly suggesting that autophosphorylation occurs between two tetramers (*trans*-autophosphorylation). A molecular model demonstrating that two tetramers can be successfully docked to carry out reciprocal *trans*-autophosphorylation has been generated by molecular dynamic relaxation of a manually docked complex in which one β subunit of each tetramer is productively engaged with a catalytic subunit of the other [38]. We asked whether a similar docking can be propagated to generate a linear polymer consistent with the known properties of CK2 filaments defined biochemically.

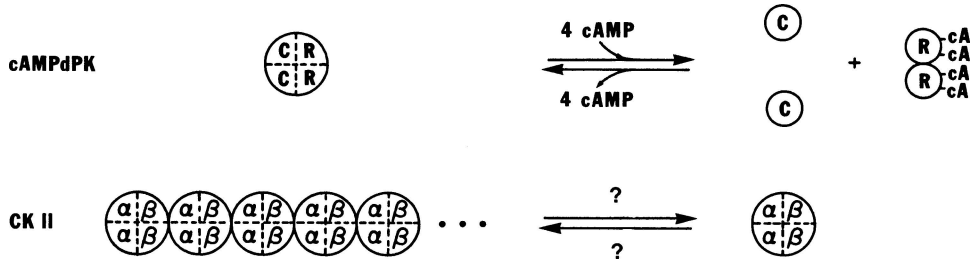

Fig. 1. An early model of CK2 regulation. Drawn in 1986, this diagram was intended to be the final figure in the paper reporting the discovery of filamentous CK2 [3] but was eliminated during review. *Top*: Inactive tetrameric cAMPdPK is dissociated upon binding of cAMP to yield two active catalytic monomers and an R_2 dimer. *Bottom*: By analogy, inactive CK2 filaments are dissociated *via* an unknown mechanism to yield active CK2 tetramers. The interaction between adjacent tetramers in this model differs in detail from that presented in Fig. 2, but the regulatory import of the two models is identical.

The human CK2 crystal structure contains an entire $\alpha_2\beta_2$ tetramer in the asymmetric unit, and hence the two $\alpha\beta$ dimers of the tetramer are not related by a crystallographic axis. Because of crystal packing forces within the P63 symmetry of the crystal, the two dimers are significantly different, the two catalytic subunits in particular differing dramatically in conformation, one being open and binding the ATP analog AMPPNP, the other closed. Because the enzyme is presumably symmetric in solution and in order to construct a uniform filament, we generated a symmetric version of the human CK2 holoenzyme as described in "Materials and methods". In this structure, both α subunits are identical, as are both β subunits. In particular, both α subunits now contain the ATP analog, and both β subunits have an N-terminal extension that includes the autophosphorylation site at Ser2. Strictly speaking, this tetramer is only approximately symmetrical as the tetramer has a slight kink at the (pseudo) twofold axis. This kink is in the original structure (as noted, the twofold axis of the tetramer does not correspond with a crystallographic axis) and is presumably due to crystal packing forces.

Two copies of this symmetric tetramer were then manually docked so as to juxtapose an autophosphorylation site on one tetramer with a catalytic site on the other, and *vice versa*. The docking geometry that is achieved is sensitive to the conformation chosen for the N-terminal arm of the β subunit and probably also to the conformation of the acidic loop, though the latter variable was not investigated. Both of these regions are disordered in the structure of the free human β dimer [39], and their visibility in the holoenzyme structure likely stems from stabilizing interactions with adjacent tetramers; both regions are presumably flexible within the context of the tetramer in solution. Nevertheless, diverse conformations of the N-terminal arm are consistent with a successful docking, that is, one in which the sidechain of Ser2 is positioned to make a nucleophilic attack on the terminal phosphoryl group of the bound nucleotide. In all of these variants, the N-terminal arm and acidic loop are closely apposed to the basic regions of the active site allowing numerous favorable charge–charge interactions, as discussed by Niefind *et al.* [36].

Each docking geometry was then propagated as described in "Materials and methods" to generate a continuous filament. A large range in the initial docking is compatible with the generation of a continuous, uniform helical filament free of steric hindrance. The pitch and even the handedness of the filament obtained is dependent upon the geometry of the initial docking, but the average diameter of the helix is essentially constant at 10 nm, which is the diameter observed experimentally in electron micrographs of negatively stained preparations [3]. An example of a pentamer constructed from the symmetric human CK2 holoenzyme is shown in Fig. 2. Because of the occlusion of active sites by autophosphorylation sites within the body of the filament, even this degree of polymerization is predicted to attenuate CK2 activity against exogenous substrates by fivefold (80% inhibition). Given the degree of polymerization observed *in vitro*, attenuation of activity by 100- to 1000-fold is possible by this mechanism [3], even if rings are excluded from consideration.

To determine whether similar filaments could be constructed for other species, we employed the symmetric human tetramer to construct homology models of Drosophila CK2

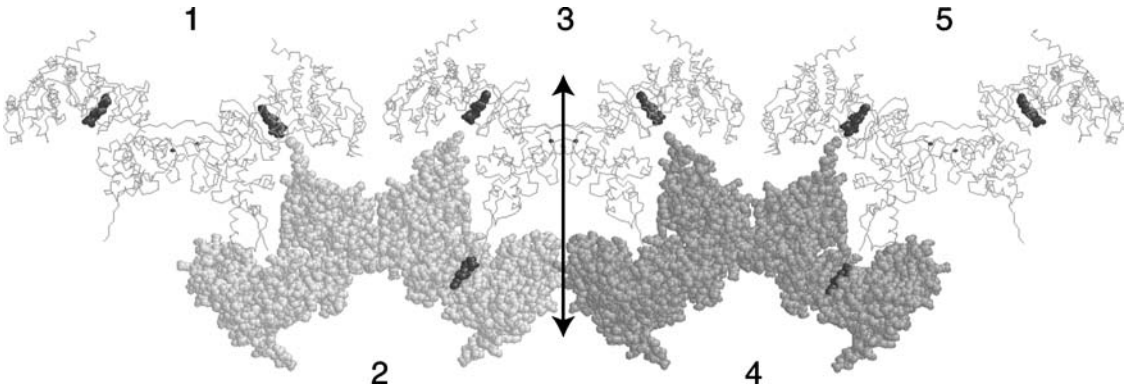

Fig. 2. Model of filamentous CK2. Beginning with the X-ray structure of human CK2 holoenzyme [36], Swiss-PdbViewer was used to generate a symmetric CK2 tetramer. Two copies of this symmetric tetramer were then manually docked to generate a dimer of tetramers, and this docking geometry was propagated to generate a short filament of five tetramers. Odd-numbered tetramers are displayed in backbone and even-numbered tetramers in spacefill (with different shading). Except where obscured by protein, AMPPNP in the active site of the α subunit and the zinc atoms of the β subunit zinc fingers are displayed in spacefill. Autophosphorylation sites and active sites are productively engaged within the filament, with each tetramer contributing one autophosphorylation site and one active site to each interface. One free active site and one free autophosphorylation site thus remain unoccupied at each end of the filament. The width of the modeled filament is approximately 10 nm (indicated by the double-headed arrow), consistent with the 10 nm diameter estimated from negatively stained images of Drosophila CK2 filaments [3]. The structure is helical but the pitch is slight and only weakly evident in the figure.

and *S. cerevisiae* CK2, the latter as an α–β–β'–α' heterotetramer [5]. We were able to generate comparable filaments free of steric hindrance in these cases as well. Although we were unable to model the large inserts that are present in the Cka1 and Ckb1 subunits of yeast CK2, these inserts enter and leave these subunits at points that are substantially exposed to solvent in the modeled structure of the yeast filament.

The astute reader will have noticed that the diagram in Fig. 1 differs from the molecular model in Fig. 2 in that the former filament is polar, whereas the latter is symmetric. Although both were logically possible *a priori*, the docking geometry shown in Fig. 1 is incompatible with the structure of the human CK2 holoenzyme as determined by X-ray crystallography.

Given that filaments are readily dissociated by salt, they are presumably held together exclusively by ionic bonds between the N-terminal arm and acidic loop on the one hand and the active site on the other. If the arm and loop are flexible, then flexibility in the geometry of the interaction is expected.

Discussion

Data mining of available bioinformatic resources has been used to explore the global physiological role of CK2 in *S. cerevisiae*. The enzyme is expressed constitutively at a modest level, is predominantly nuclear, is a component of several nuclear, chromatin-associated complexes, and is predicted to phosphorylate a broad spectrum of nuclear as well as cytoplasmic proteins involved in all aspects of gene expression. In addition, transcriptional profiling indicates that deletion of individual CK2 subunits results in changes in the expression of a number of chromatin remodeling activities. While doubtless an oversimplification, the combined results suggest an important role for CK2 in some aspect of chromatin structure and dynamics and/or global gene expression. A recent bioinformatic compilation of global functional genomics data available in *S. cerevisiae* has yielded a similar conclusion: Cka1, Ckb1, and Ckb2 cluster with proteins categorized as involved in transcription, translation, and RNA processing, Cka2 with proteins involved in cell cycle and DNA processing [40].

Because of the intimate association between the mechanism of regulation of a protein kinase and its physiological role, further progress in defining the global function of CK2 is unlikely until its mechanism of regulation (assuming that it has one) is understood. Inspired by the recent crystal structure of the CK2 holoenzyme, we have reexamined the earlier proposal [3] that filamentous CK2 represents an inactive form of CK2 and that reversible polymerization/depolymerization may thus hold the key to its intracellular regulation. Consistent with this proposal, we show here that it is possible to construct a molecular model of filamentous CK2, consistent with available constraints, in which the catalytic sites of the α subunits within the polymer are occluded by interaction with the autophosphorylation sites of the β subunits. This model predicts that filamentous CK2 should be essentially inert toward external substrates but potentially capable of autophosphorylation. Biochemical data in support of this contention have recently been obtained by Meggio, who has demonstrated that there exists a strict ionic-strength-dependent correlation between autophosphorylation, phosphorylation of exogenous substrates, and the polymeric state of the enzyme: autophosphorylation is prominent under conditions of low ionic strength where the enzyme is oligo- or polymeric but essentially absent at high salt where the kinase is tetrameric, while activity toward exogenous substrates behaves reciprocally [37]. These data are consistent with the molecular model of filamentous CK2 presented here and provide strong biochemical support for the proposition that polymeric CK2 represents an inactive form of the enzyme.

Do filaments exist *in vivo*? There are currently no direct data bearing on this issue. However, recent analysis of cells expressing fluorescent derivatives of CK2 subunits *via* fluorescence recovery after photobleaching has identified two pools of CK2, one with a fast mobility *in vivo* and the other with a slow mobility [41]. Filaments represent one possible explanation for the slow pool, but other explanations are not excluded, such as tethering of tetramers to the cytoskeleton or other immobilized structures. That CK2 is extensively autophosphorylated as obtained from cells provides perhaps the strongest current argument: if it is indeed true that autophosphorylation cannot occur in *cis*, then at least transient formation of higher-order oligomers *in vivo* is a logical necessity.

Collectively, the above results provide significant new support for the proposition that CK2 activity *in vivo* is regulated by reversible polymerization/depolymerization of the enzyme. If this proposition is correct, two important questions remain: what signal(s) regulates the holoenzyme–polymer equilibrium and hence enzyme activity, and to what end? Because CK2 polymerization is strongly affected by ionic strength, one possibility is that the enzyme responds directly to the ionic milieu of the cell, providing a potential sensing mechanism that could be used to initiate necessary cellular responses to ionic perturbations [5]. Both chromatin structure and gene expression involve extensive interactions of polyanions (DNA and RNA) with polycations (histones, ribonucleoprotein particle proteins, ribosomal proteins), and such interactions are strongly dependent on an appropriate ionic environment. CK2 could provide a mechanism to buffer such crucial cellular machinery from changes in intracellular ionic conditions, *via* either of two mechanisms. On the one hand, CK2 might act to restore the normal ionic equilibrium; on the other, CK2 might phosphorylate diverse targets within the cell in order to allow the gene expression machinery to

tolerate the adverse ionic conditions. Experimental support for this scenario is provided by the fact that yeast strains carrying hypomorphic mutations in CK2 display increased sensitivity to Na^+ and Li^+ [5, 9], and this sensitivity has been explained by both mechanisms described above [42, 43]. While the two mechanisms are not incompatible, the second [43] is of particular interest, as it offers a potential explanation for the extraordinarily broad substrate specificity of CK2 [1]. Other potential regulators include low-molecular-weight compounds such as polyamines [4] or yet to be identified protein regulators.

CK2 is commonly regarded as a constitutively active protein kinase that is messenger independent. Recent developments suggest that it is time to reconsider the alternative, namely, that CK2 is a constitutively inactive enzyme, by virtue of a default formation of inactive filaments. If so, identification of potential regulators of the tetramer–filament equilibrium becomes a high priority. The ionic milieu itself represents one intriguing possibility.

An obvious need is for a method to monitor CK2 polymerization in living cells so that the existence of filaments can be confirmed *in vivo* and their behavior monitored in response to developmental and environmental cues or genetic perturbations. Molecular models of polymeric CK2 such as that proposed here should facilitate the rational design of appropriate fusion constructs for this purpose.

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References

1. Meggio F, Pinna LA: One-thousand-and-one substrates of protein kinase CK2? FASEB J 17: 349–368, 2003
2. Litchfield DW: Protein kinase CK2: structure, regulation and role in cellular decisions of life and death. Biochem J 369: 1–15, 2003
3. Glover CV: A filamentous form of Drosophila casein kinase II. J Biol Chem 261: 14349–14354, 1986
4. Valero E, De Bonis S, Filhol O, Wade RH, Langowski J, Chambaz EM, Cochet C: Quaternary structure of casein kinase 2. Characterization of multiple oligomeric states and relation with its catalytic activity. J Biol Chem 270: 8345–8352, 1995
5. Glover CV, 3rd: On the physiological role of casein kinase II in Saccharomyces cerevisiae. Prog Nucleic Acid Res Mol Biol 59: 95–133, 1998
6. Padmanabha R, Chen-Wu JL, Hanna DE, Glover CV: Isolation, sequencing, and disruption of the yeast *CKA2* gene: casein kinase II is essential for viability in Saccharomyces cerevisiae. Mol Cell Biol 10: 4089–4099, 1990
7. Hanna DE, Rethinaswamy A, Glover CV: Casein kinase II is required for cell cycle progression during G1 and G2/M in Saccharomyces cerevisiae. J Biol Chem 270: 25905–25914, 1995
8. Rethinaswamy A, Birnbaum MJ, Glover CV: 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: 5869–5877, 1998
9. Bidwai AP, Reed JC, Glover CV: Cloning and disruption of *CKB1*, the gene encoding the 38-kDa beta subunit of Saccharomyces cerevisiae casein kinase II (CKII). Deletion of CKII regulatory subunits elicits a salt-sensitive phenotype. J Biol Chem 270: 10395–10404, 1995
10. Toczyski DP, Galgoczy DJ, Hartwell LH: *CDC5* and CKII control adaptation to the yeast DNA damage checkpoint. Cell 90: 1097–1106, 1997
11. Bandhakavi S, McCann RO, Hanna DE, Glover CV: Genetic interactions among *ZDS1,2*, *CDC37*, and protein kinase CK2 in Saccharomyces cerevisiae. FEBS Lett 554: 295–300, 2003
12. Bandhakavi S, McCann RO, Hanna DE, Glover CV: A positive feedback loop between protein kinase CKII and Cdc37 promotes the activity of multiple protein kinases. J Biol Chem 278: 2829–2836, 2003
13. Cardenas ME, Dang Q, Glover CV, Gasser SM: Casein kinase II phosphorylates the eukaryote-specific C-terminal domain of topoisomerase II *in vivo*. EMBO J 11: 1785–1796, 1992
14. Feng L, Yoon H, Donahue TF: Casein kinase II mediates multiple phosphorylation of Saccharomyces cerevisiae eIF-2 alpha (encoded by *SUI2*), which is required for optimal eIF-2 function in *S. cerevisiae*. Mol Cell Biol 14: 5139–5153, 1994
15. Ghavidel A, Schultz MC: TATA binding protein-associated CK2 transduces DNA damage signals to the RNA polymerase III transcriptional machinery. Cell 106: 575–584, 2001
16. Miyata Y, Nishida E: CK2 controls multiple protein kinases by phosphorylating a kinase-targeting molecular chaperone, Cdc37. Mol Cell Biol 24: 4065–4074, 2004
17. Bader GD, Heilbut A, Andrews B, Tyers M, Hughes T, Boone C: Functional genomics and proteomics: charting a multidimensional map of the yeast cell. Trends Cell Biol 13: 344–356, 2003
18. Hulo N, Sigrist CJ, Le Saux V, Langendijk-Genevaux PS, Bordoli L, Gattiker A, De Castro E, Bucher P, Bairoch A: Recent improvements to the PROSITE database. Nucleic Acids Res 32: D134–137, 2004
19. Obenauer JC, Cantley LC, Yaffe MB: Scansite 2.0: proteome-wide prediction of cell signaling interactions using short sequence motifs. Nucleic Acids Res 31: 3635–3641, 2003
20. Schwede T, Kopp J, Guex N, Peitsch MC: SWISS-MODEL: an automated protein homology-modeling server. Nucleic Acids Res 31: 3381–3385, 2003
21. Ficarro SB, McCleland ML, Stukenberg PT, Burke DJ, Ross MM, Shabanowitz J, Hunt DF, White FM: Phosphoproteome analysis by mass spectrometry and its application to Saccharomyces cerevisiae. Nat Biotechnol 20: 301–305, 2002
22. Uetz P, Giot L, Cagney G, Mansfield TA, Judson RS, Knight JR, Lockshon D, Narayan V, Srinivasan M, Pochart P, Qureshi-Emili A, Li Y, Godwin B, Conover D, Kalbfleisch T, Vijayadamodar G, Yang M, Johnston M, Fields S, Rothberg JM: A comprehensive analysis of protein–protein interactions in Saccharomyces cerevisiae. Nature 403: 623–627, 2000
23. Gavin AC, Bosche M, Krause R, Grandi P, Marzioch M, Bauer A, Schultz J, Rick JM, Michon AM, Cruciat CM, Remor M, Hofert C, Schelder M, Brajenovic M, Ruffner H, Merino A, Klein K, Hudak M, Dickson D, Rudi T, Gnau V, Bauch A, Bastuck S, Huhse B, Leutwein C, Heurtier MA, Copley RR, Edelmann A, Querfurth E, Rybin V, Drewes G, Raida M, Bouwmeester T, Bork P, Seraphin B, Kuster B, Neubauer G, Superti-Furga G: Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature 415: 141–147, 2002
24. Hazbun TR, Malmstrom L, Anderson S, Graczyk BJ, Fox B, Riffle M, Sundin BA, Aranda JD, McDonald WH, Chiu CH, Snydsman BE, Bradley P, Muller EG, Fields S, Baker D, Yates JR, 3rd, Davis TN:

Assigning function to yeast proteins by integration of technologies. Mol Cell 12: 1353–1365, 2003
25. Krogan NJ, Kim M, Ahn SH, Zhong G, Kobor MS, Cagney G, Emili A, Shilatifard A, Buratowski S, Greenblatt JF: RNA polymerase II elongation factors of Saccharomyces cerevisiae: a targeted proteomics approach. Mol Cell Biol 22: 6979–6992, 2002
26. Hughes TR, Marton MJ, Jones AR, Roberts CJ, Stoughton R, Armour CD, Bennett HA, Coffey E, Dai H, He YD, Kidd MJ, King AM, Meyer MR, Slade D, Lum PY, Stepaniants SB, Shoemaker DD, Gachotte D, Chakraburtty K, Simon J, Bard M, Friend SH: Functional discovery via a compendium of expression profiles. Cell 102: 109–126, 2000
27. Peng WT, Robinson MD, Mnaimneh S, Krogan NJ, Cagney G, Morris Q, Davierwala AP, Grigull J, Yang X, Zhang W, Mitsakakis N, Ryan OW, Datta N, Jojic V, Pal C, Canadien V, Richards D, Beattie B, Wu LF, Altschuler SJ, Roweis S, Frey BJ, Emili A, Greenblatt JF, Hughes TR: A panoramic view of yeast noncoding RNA processing. Cell 113: 919–933, 2003
28. Barz T, Ackermann K, Dubois G, Eils R, Pyerin W: Genome-wide expression screens indicate a global role for protein kinase CK2 in chromatin remodeling. J Cell Sci 116: 1563–1577, 2003
29. Tong AH, Lesage G, Bader GD, Ding H, Xu H, Xin X, Young J, Berriz GF, Brost RL, Chang M, Chen Y, Cheng X, Chua G, Friesen H, Goldberg DS, Haynes J, Humphries C, He G, Hussein S, Ke L, Krogan N, Li Z, Levinson JN, Lu H, Menard P, Munyana C, Parsons AB, Ryan O, Tonikian R, Roberts T, Sdicu AM, Shapiro J, Sheikh B, Suter B, Wong SL, Zhang LV, Zhu H, Burd CG, Munro S, Sander C, Rine J, Greenblatt J, Peter M, Bretscher A, Bell G, Roth FP, Brown GW, Andrews B, Bussey H, Boone C: Global mapping of the yeast genetic interaction network. Science 303: 808–813, 2004
30. Huh WK, Falvo JV, Gerke LC, Carroll AS, Howson RW, Weissman JS, O'Shea EK: Global analysis of protein localization in budding yeast. Nature 425: 686–691, 2003
31. Krek W, Maridor G, Nigg EA: Casein kinase II is a predominantly nuclear enzyme. J Cell Biol 116: 43–55, 1992
32. Holstege FC, Jennings EG, Wyrick JJ, Lee TI, Hengartner CJ, Green MR, Golub TR, Lander ES, Young RA: Dissecting the regulatory circuitry of a eukaryotic genome. Cell 95: 717–728, 1998
33. Ghaemmaghami S, Huh WK, Bower K, Howson RW, Belle A, Dephoure N, O'Shea EK, Weissman JS: Global analysis of protein expression in yeast. Nature 425: 737–741, 2003
34. Pinna LA: Protein kinase CK2: a challenge to canons. J Cell Sci 115: 3873–3878, 2002
35. Faust M, Montenarh M: Subcellular localization of protein kinase CK2. A key to its function? Cell Tissue Res 301: 329–340, 2000
36. Niefind K, Guerra B, Ermakowa I, Issinger OG: Crystal structure of human protein kinase CK2: insights into basic properties of the CK2 holoenzyme. EMBO J 20: 5320–5331, 2001
37. Pagano MA, Sarno S, Poletto G, Cozza G, Pinna LA, Meggio F: Autophosphorylation at the regulatory β subunit reflects the supramolecular organization of protein kinase CK2. Mol Cell Biochem 274: 23–29, 2005
38. Rekha N, Srinivasan N: Structural basis of regulation and substrate specificity of protein kinase CK2 deduced from the modeling of protein-protein interactions. BMC Struct Biol 3: 4, 2003
39. Chantalat L, Leroy D, Filhol O, Nueda A, Benitez MJ, Chambaz EM, Cochet C, Dideberg O: Crystal structure of the human protein kinase CK2 regulatory subunit reveals its zinc finger-mediated dimerization. EMBO J 18: 2930–2940, 1999
40. Lee I, Date SV, Adai AT, Marcotte EM: A probabilistic functional network of yeast genes. Science 306: 1555–1558, 2004
41. Theis-Febvre N, Martel V, Laudet B, Souchier C, Grunwald D, Cochet C, Filhol O: Highlighting protein kinase CK2 movement in living cells. Mol Cell Biochem 274: 15–22, 2005
42. Tenney KA, Glover CV: Transcriptional regulation of the *S. cerevisiae ENA1* gene by casein kinase II. Mol Cell Biochem 191: 161–167, 1999
43. de Nadal E, Calero F, Ramos J, Arino J: Biochemical and genetic analyses of the role of yeast casein kinase 2 in salt tolerance. J Bacteriol 181: 6456–6462, 1999