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Promiscuous Subunit Interactions: A Possible Mechanism for the Regulation of Protein Kinase CK2

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Abstract Protein kinase CK2 is a ubiquitous eukaryotic ser/thr protein kinase. The active holoenzyme is a heterotetrameric protein composed of catalytic (α and α′) and regulatory (β) subunits that phosphorylates many different protein substrates and appears to be involved in the regulation of cell division. Despite important structural studies, the intimate details of the interactions of the α catalytic subunits with the β regulatory subunits are unknown. Recent evidence that indicates that both CK2 subunits can interact promiscuously with other proteins in a manner that excludes the binding of their complementary CK2 partners has opened the possibility that the phosphorylating activity of this enzyme may be regulated in a novel way. These alternative interactions could limit the in vivo availability of CK2 subunits to generate fully active holoenzyme CK2 tetramers. Likewise, variations in the ratio of α- and β-subunits could determine the activity of several phosphorylating and dephosphorylating activities. The promiscuity of the CK2 subunits can be extrapolated to a more widespread phenomenon in which "wild-card" proteins could act as general switches by interacting and regulating several catalytic activities. J. Cell. Biochem. Suppls. 30/31:129–136, 1998. © 1998 Wiley-Liss, Inc.

Key words: protein kinase CK2; holoenzyme; α- and β-subunits

Protein kinase CK2 (previously known as casein kinase II) is ubiquitous in eukaryotes and is responsible for the phosphorylation of a large number of protein substrates [for reviews, see Allende and Allende, 1995; Issinger, 1993; Pinna and Meggio, 1997]. The active holoenzyme is a heterotetramer composed of catalytic subunits (α and α′), which are coded by different but highly analogous genes, and of regulatory subunits (β-subunits). In yeast, there are two different β-subunits, while in some plants and in *Dictyostelium,* no β-subunits have been found as part of active CK2. Different combinations of these subunits ($\alpha_2\beta_2$, $\alpha\alpha'\beta_2$ and $\alpha'_2\beta_2$) have been detected in mammals. The isolated α and α′ subunits are monomeric and are active by themselves, but their catalytic activity can be greatly modified, but the presence of the β-subunits to which they bind with high affinity. The isolated β-subunits form dimers that are responsible for the assembly of the tetrameric structure. The effect of β on the phosphorylation activity of α depends on the protein substrate. With most known protein substrates and with model peptides, β causes a stimulation of the catalytic activity of 5- to 20-fold, while with a few other substrates, such as calmodulin and MDM-2 [Bidwai et al., 1993; Guerra et al., 1997], the regulatory subunit strongly inhibits the phosphorylating capacity of α and α′.

Amino acid sequence comparisons of the catalytic domains of eukaryotic protein kinases [Hanks and Hunter, 1995] permit the grouping together of protein kinases in a dendrogram based on the use of sequence similarities reflecting evolutionary relatedness. Interestingly, these groups also display functional similarities. The α-subunit of protein kinase CK2 belongs to the "CMGC group," which also includes the large family of cyclin-dependent protein kinases (CDKs), the mitogen-activated protein kinases (MAPKs or ERKs), and glycogen syn-

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thase kinase-3. The presence of CK2α in this group with other kinases involved in the regulation of cell division suggests that this enzyme may also have a similar function. This suggestion is borne out by abundant experimental evidence. For example, the amount and activity of CK2 is higher in dividing and transformed cells than in resting cells [Issinger, 1993]. CK2α expressed in transgenic animals under the control of an immunoglobulin promoter can act as an oncogene that complements *myc* in the generation of lymphomas [Seldin and Leder, 1995]. Reduction of the α- or β-subunit levels through antisense treatment of cells in culture or through antibody microinjection causes the cells to stop in the G_1/S and G_2/M transition point of the cell cycle [Pepperkok et al., 1994]. A similar effect has also been obtained with the use of temperature-sensitive mutants of the α-subunits of *Saccharomyces cerevisiae* [Rethinaswamy et al., 1998]. In prostate gland cells that have been stimulated to grow and divide by testosterone treatment [Tawfic and Ahmed, 1994]. CK2 activity increases and the enzyme translocates into the nucleus. Finally, CK2 is known to phosphorylate many proteins that have important functions in cell division [Allende and Allende, 1995; Pinna and Meggio, 1997].

There are additional interesting and important similarities between CK2 and the CDKs, especially CDK2. CK2 has only 33% identity with CDK2, but when conservative amino acid substitutions are considered, the similarity goes up to 60%. The two types of kinases share the property of having regulatory subunits, cyclins for CDKs and β-subunit for CK2, which increase catalytic activity with most substrates. These regulatory subunits contain a "destruction box" sequence that may signal their degradation by the ubiquitin pathway [Allende and Allende, 1995]. As we shall see, this similarity between cyclins and the CK2β subunits is also recognized by $p21^{WAF1/CIP1}$, which binds both types of regulators [Götz et al., 1996].

STRUCTURAL FEATURES AND THE PROBLEM OF THE INTERACTION BETWEEN THE α- AND β-SUBUNITS

A number of studies have been carried out on structural aspects of CK2 function. Using information about the sequences phosphorylated in native proteins, a large battery of peptides have been tested to determine the minimum consensus sequence recognized by CK2, which is S*/T*XXE/D, in which S*/T* denotes the serine or threonine phosphorylated and E/D either glutamic or aspartic acid in the third position toward the carboxyl end [Pinna and Meggio, 1997]. The third position acidic residue can be replaced by a phosphoamino acid and often the preferred substrate sequences have additional acidic groups upstream and downstream from the target serine and threonine. Site-directed mutants have been designed to determine the residues in the CK2α subunit that are involved in the recognition of the peptide substrates [Pinna and Meggio, 1997]. Similar efforts have been carried out to explore the structural basis for the unique capacity of CK2α to use GTP, as well as ATP, as a phosphate donor [Jakobi and Traugh, 1995; Srinivasan et al., 1999]. The resolution of the crystal structure of zea maize CK2α by X-ray diffraction constitutes a major contribution to our understanding of some of the catalytic properties of this enzyme [Niefind et al., 1998].

An important aspect that still requires further work is the nature of the interactions between the α- and β-subunits. It is clear that these two subunits bind each other with great affinity, as their dissociation requires treatment with strong denaturing reagents [Cochet and Chambaz, 1983], and their association occurs spontaneously, even at zero degrees. Site-directed mutagenesis, the yeast-two hybrid system, and cross-linking studies indicate that the β-subunit has two regions that interact with CK2α. The carboxyl region of CK2β, including amino acids 155–190, is essential for its binding to CK2α and the protection of α against thermal inactivation. In addition, this region is also responsible for the increase in its catalytic activity. Within this region (amino acids 155–167) is a sequence involved in β/β dimer formation [Pinna and Meggio, 1997]. There is no study, however, that provides significant information about the region(s) of CK2α that interact(s) directly with the carboxyl activating region of CK2β.

The second region of the β-subunit that interacts with α is the highly acidic region located at amino acids 55–64 (DLEPDEELED), which appears to interact with a basic region of α (amino acids 74–83, KKKKIKREIK). The acidic region of β causes a decrease in the catalytic activity of α-subunit, as mutation of these acidic residues or of proline 58 to alanine results in an increase

in the capacity of CK2β to activate the phosphorylation of protein substrates. The same acidic region of β is responsible for the capacity of polycations such as spermine or polylysine to activate CK2. Serines 2 and 3 in the β-subunit are phosphorylated by α, but their elimination by mutagenesis does not seem to affect significantly subunit interaction or change the effects of β on α catalysis [Allende and Allende, 1995; Pinna and Meggio, 1997]. The lysine-rich region of CK2α (K^{74}-K^{83}), which interacts with the acidic region of β and results in downregulation of catalytic activity is also responsible for the highly inhibitory effect of polyanions such as heparin, poly(glutamic-tyrosine) peptides, and nucleic acids [Allende and Allende, 1995; Pinna and Meggio, 1997]. This basic region also has the structural requirements of a nuclear localization sequence. A model of a possible conformation of $\alpha_2\beta_2$ tetramer of CK2 that accounts for the known interactions and effects of β-subunit on CK2α is shown in Figure 1.

An inactive mutant of CK2α (CK2α^{A156}) that binds β with high affinity [Cosmelli et al., 1997],

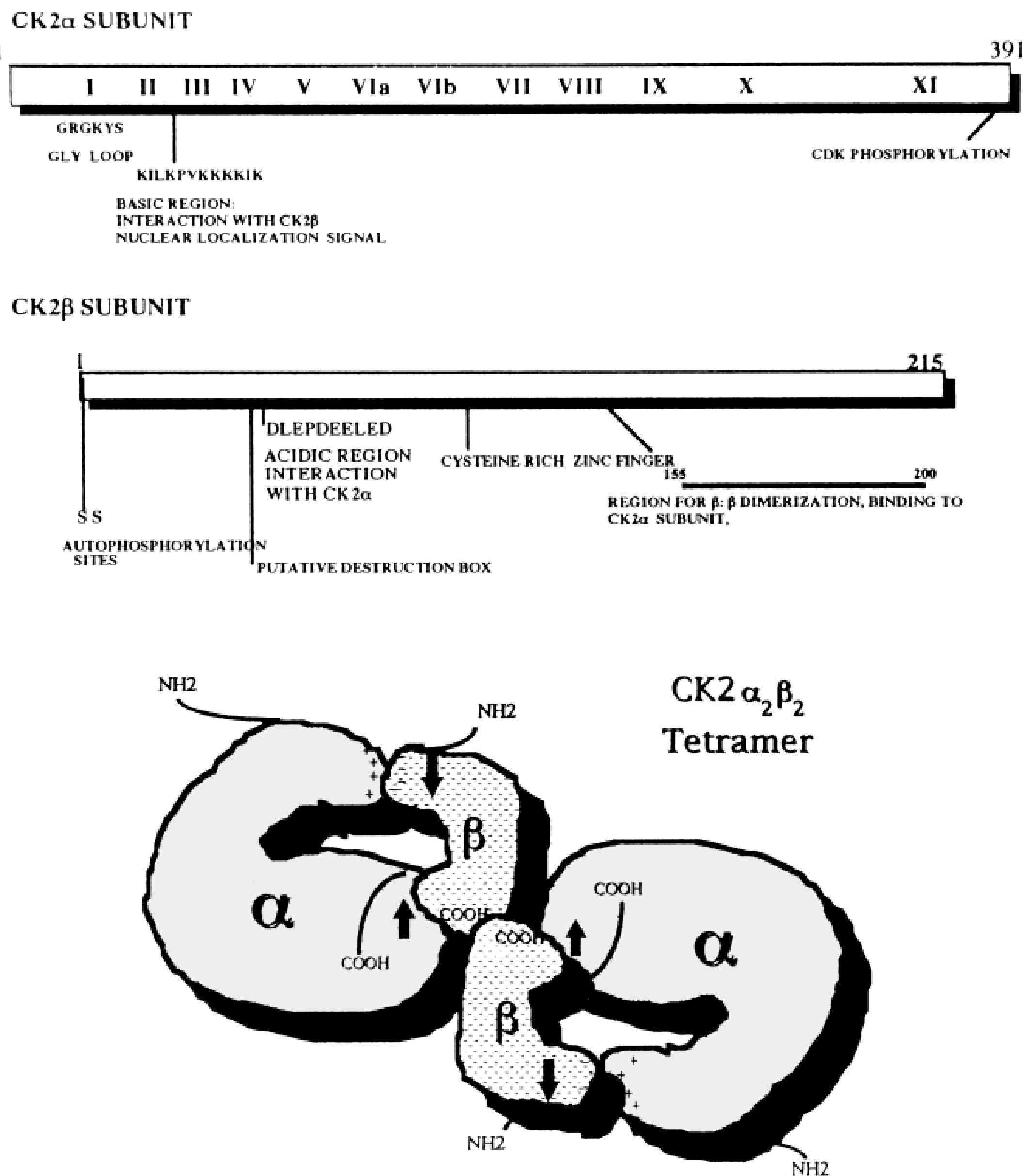


Fig. 1. **A:** Schematic representation of some distinctive structural features in CK2α- and β-subunits. **B:** The tetrameric CK2$\alpha_2\beta_2$ holoenzyme. The + and − signs indicate positively and negatively charged regions in the subunits. The effect of subunit interaction on catalytic activity (with most CK2 subunits) is indicated by *arrows:* activation (↑), attenuation (↓).

provides us with a tool to measure relative affinities of active forms of the catalytic subunit, as CK2α^{A156} competes for β and traps it in an inactive complex. Under conditions of limiting β concentrations, the addition of increasing concentration of CK2α^{A156} causes a very similar gradual loss of the stimulatory effect of β on the catalytically active forms of CK2α and α', indicating that both α-subunits have very similar affinity to β. A mutant of CK2α of *Xenopus* (CK2α^{E75E76}) was also tested recently in this competition assay (Allende, J.E., unpublished observations). This mutant is fully active but has an altered basic region by the introduction of two glutamic acid residues that replace lysines. This mutation greatly reduces the inhibition by heparin [Pinna and Meggio, 1997] and might be predicted to eliminate or greatly reduce the ionic interaction with the acidic stretch of β. The results obtained, however, show that the mutation of two lysine residues CK2α^{E75E76} does not affect the overall α–β interaction, indicating that this ionic interaction of the subunits is probably hydro-subordinated to a much stronger phobic interaction between an undetermined CK2α region and the carboxyl region of CK2β.

THE ENIGMA OF THE REGULATION OF CK2

The role that CK2 appears to play in the process of cell division generates considerable interest as to the nature of the regulation of CK2α and β expression. Although precise mechanisms of regulation have not been defined, accumulating evidence supports that there exists long-term regulation of CK2 gene expression through a tight control of mRNA levels and protein kinase activity. There are differences in the amount of mRNA coding for these subunits in various tissues [Issinger, 1993]. An example of this differential expression can be detected by in situ hybridization of mRNAs in developing embryos, in which higher concentrations of mRNAs for CK2 are observed in nervous tissues [Daniotti et al., 1994]. Also, the amount of CK2 mRNAs and proteins in tumors and transformed cells has been found to be higher than in normal tissues [Issinger, 1993]. Pyerin's group has reported that free α-subunit can stimulate the transcription of β-subunit [Robitzki et al., 1993]. Orlandini et al. [1998] recently reported that serum can stimulate the transcription of the α' and other CK2 subunits in quiescent cells. The factors responsible for this transcriptional regulation remain unknown.

Post-translational modifications of CK2 subunits as a mechanism of regulating activity have not been reproducibly demonstrated. This is the conclusion of Lichtfield et al. [1994], after careful re-examination of the conflicting evidence published some time ago. Very recently, however, Hériché and Chambaz [1998] have reported that c-Abl can phosphorylate CK2α on tyrosine and inhibit its activity. This observation is noteworthy because CK2 has a tyrosine residue in position 49 of the glycine-rich loop of conserved region I. This tyrosine is the equivalent of tyrosine 15 of cdc-2, which is phosphorylated by the wee kinase, causing an inhibition of the enzyme and constituting an important regulatory mechanism in cell cycle progression.

Specific proteolytic degradation can constitute a regulatory mechanism, as seen with the cyclins and CDKs. As mentioned previously, the presence of a "destruction box"-like sequence in the β-subunit suggests that ubiquitin pathway degradation may also regulate the levels of this subunit. Lüscher and Lichtfield [1994] have found that β is synthesized in a large excess over α and also that it is degraded much more rapidly than the catalytic subunit.

Another regulatory mechanism that appears to operate in the case of CK2 is that of intracellular translocation. In quiescent cells, the enzyme is located mainly in the cytosol while dividing cells concentrate CK2 in the nucleus. This phenomenon is particularly striking in prostate tissue before and after stimulation with androgenic hormones [Tawfic and Ahmed, 1994].

Several protein kinases are regulated by specific protein inhibitors. This is the case with cyclic adenosine monophosphate (cAMP)-dependent protein kinase, which is inactivated by PKI [Walsh et al., 1971] and with the CDKs, in which a whole family of inhibitors (CKIs) have been detected [Morgan, 1995]. These inhibitors are of great interest because of their role in regulating the cell cycle and because their alteration through mutations may lead to tumorigenesis. p21$^{WAF1/CIP1}$ is a potent general inhibitor of CDK's and mediates some of the tumor suppressor effects of p53, which enhances the transcription of this inhibitory polypeptide. The structural and functional similarities of CK2 and CDK2 prompted Götz et al. [1996] to test the interaction of p21$^{WAF1/CIP1}$ with CK2. It was

demonstrated that p21 binds to the β-subunit of CK2 causing an inhibition of the phosphorylating activity of the CK2 holoenzyme. Further, it has been demonstrated in our laboratory that CK2 subunits can interact with $p21^{WAF1/CIP1}$ in transfected cos-7 cells [Korn et al., 1998]. These findings suggest that $p21^{WAF1/CIP1}$ may act as a physiological regulator of CK2 and that it may play a more general role as an inhibitor of kinases that are involved in driving forward the cell cycle. In this regard, it is relevant that $p21^{WAF1/CIP1}$ has also been found to inhibit stress-activated kinases [Shim et al., 1996].

There has been a good deal of debate about whether the CK2β subunit is a true physiological regulator of CK2 activity or whether it is a mere constitutive partner of the holoenzyme, without true regulatory capacity. The notion of constituency is supported by the fact that CK2α is found in animal tissues already bound to stochiometric amounts of β, whereas isolated subunits are not readily detected in tissue extracts and no mechanism has been found to permit the dissociation of the two subunits, except through the use of drastic denaturating agents [Cochet and Chambaz, 1983]. There are, however, reports of CK2α subunit bound to chromatin in the absence of β [Stigare et al., 1993]. Gatica et al. [1994] found that α can bind DNA unspecifically but that the addition of β causes the release of the enzyme from the nucleic acid.

The excess of CK2β over CK2α in some cells may be accounted for by its interaction with other proteins (see below), as free CK2β is very unstable and susceptible to degradation [Lüscher and Lichtfield, 1994]. In some tumors, the amount of CK2β present indeed exceeds by far the amount of α-subunit [Pinna and Meggio, 1997]. By contrast, transfection of β causes an increase in the CK2 activity of the extracts of COS-1 cells [Heller-Harrison and Czech, 1991], a finding whose simplest interpretation is that the availability of β in these cells is a limiting factor.

PROMISCUITY OF THE CK2 SUBUNITS: INDEPENDENT INTERACTIONS WITH OTHER PROTEINS

A new vision of the α/β interplay has been provided mainly by the use of the yeast two-hybrid system, showing that these subunits have other protein partners that may compete for their reciprocal binding.

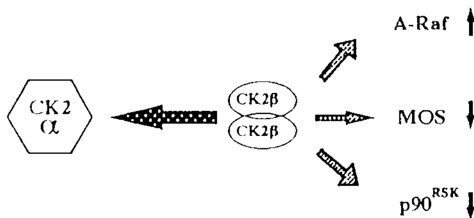
The CK2 holoenzyme has been shown to bind to many proteins in a manner that goes beyond their recognition as substrates. Examples include p53, topoisomerase IIa, nucleolin, and transcription factor ATF1 [Pinna and Meggio, 1997; Yamaguchi et al., 1998]. These interactions are interesting because of the possible effects on the targeting of either protein partner to special cellular localizations or because of the possible modifications of their properties caused by complex formations. Such regulation was recently reported [Raman et al., 1998], using the yeast two-hybrid system, which detected the interaction of CK2β subunit with the cytoplasmic domain of the transmembrane protein CD5. Immunoprecipitation of CD5 from extracts of B and T cells showed co-precipitation of CK2α. The association of CK2 subunits with CD5 is mediated by the N-terminal segment of β (2–132); it is independent of the phosphorylation of the membrane protein but dependent on the presence of CK2 phosphorylation consensus sequence in CD5. The novelty of these studies is that CK2, apparently in the holoenzyme conformation, is 10-fold stimulated by pretreatment of B lymphoma or T-leukemia cell lines with anti-CD5 antibody, without net recruitment of CK2 to CD5, indicating a direct regulation of CK2 activity by a membrane protein.

Most relevant to the present discussion, however, are the independent interactions of CK2α or β with other proteins and that exclude the binding with each other. Using the yeast two-hybrid system and recombinant CK2β as "bait," a number of interacting proteins have been detected. Boldyreff and Issinger [1997] found that β could specifically bind to the A-Raf protein. A similar interaction was obtained by Hagemann et al. [1997], using A-Raf as bait. These complementary findings indicate that CK2β binds A-Raf through a carboxyl region of CK2β very similar to the region involved in its interaction with CK2α. It is noteworthy that the interaction of A-Raf with β was lost upon mutation of a single arginine residue of A-Raf ($R^{175} \rightarrow A$), although additional amino acids downstream were also shown to be important for interaction [Boldyreff and Issinger, 1997]. These studies also showed that β can activate A-Raf phosphorylation of MEK and that the presence of CK2α blocks that effect. Almost simultaneously, another two-hybrid screen was performed with the protein kinase Mos as bait,

and again CK2β was found as the interacting partner, also binding through its carboxy region (150–215) [Chen et al., 1997]. The results show that in this case the presence of CK2β caused an inhibition of Mos activity in triggering *Xenopus* oocyte maturation. Again, CK2α was able to reverse the process, in this case the inhibitory effect of β. It is highly significant that both A-Raf and Mos are ser/thr protein kinases, which, as such, have overall structures related to CK2α and that both play a similar role in the phosphorylation of MEK in the RAS-MAPK pathway of cell division. Another kinase downstream in that pathway, $p90^{RSK}$, has also been shown to interact with and be inhibited by CK2β (B. Boldyreff, personal communication).

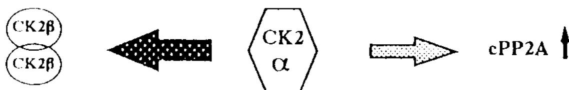
It seems, therefore, that CK2β has the potential to regulate a number of protein kinases that play important roles in cell proliferation (Fig. 2A). One aspect that requires further experimental analysis is the relative affinities of the other kinases for CK2β. If their affinities were of the same order as that of CK2α, their capacity to compete for the regulatory subunit would mean that increased synthesis of A-Raf, Mos, or $p90^{RSK}$ may cause a lowering of the cellular activity of CK2 by restricting the availability of CK2β. The results of CK2β overexpres-

A.



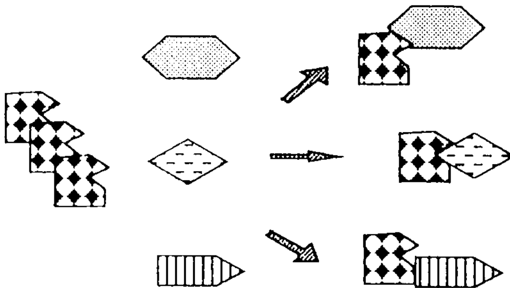
CK2β interaction with protein kinases involved in cell division: a ratio greater than one of CK2 β to α will permit regulation of the other kinases.

B.



CK2α interacts with CK2β or with protein phosphatase 2A (cPP2A): a ratio greater than one of CK2α to β would favor increased dephosphorylation reactions.

C.



"WILD CARD" proteins interact and regulate many different proteins and constitute master switches for metabolism and signal transduction.

Fig. 2. Potential regulatory mechanisms involving CK2 subunits.

sion by transfection that yielded higher CK2 activity in cell extracts [Korn et al., 1998] would be explained by this competitive interaction with other kinases. One may ask how many other kinases can interact with and be regulated by CK2β and what general features of these kinases is being recognized by this regulatory protein.

CK2β is not the only promiscuous partner in the CK2 holoenzyme. CK2α has been found to interact with the catalytic subunit of protein phosphatase 2 A (PP2A) in a manner that is competitive with CK2β [Hériché et al., 1997] (Fig. 2B). CK2α apparently binds PP2A through the sequence ^{165}DHEHRKLRLID^{175}, which is analogous to a sequence present in the small t antigen of SV-40 and which also binds the phosphatase. CK2α can phosphorylate and apparently activate PP2A.

The Janus-type complex formation between kinases and phosphatases is not unique for CK2α and PP2A. PP2A forms a stable partner in vitro with Ca^{2+}/calmodulin-dependent protein kinase IV (CamKIV) and dephosphorylates a threonine residue required for the kinase activity [Westphal et al., 1998], thus regulating the duration of an intracellular Ca^{2+} signal. A third example of such an interaction is that of MAP kinase phosphatase-3, which is activated by binding to ERK2 kinase [Camps et al., 1998].

Relevant to this discussion is the finding that CK2β subunit may be structurally related to the protein phosphatase family. The Propsearch computer program which compares proteins on the basis of several characteristics, such as amino acid pairs, composition, and pI, rather than on linear sequence, indicated a high score for overall similarity between CK2β and protein phosphatase 2A, as well as to ion-transport ATPases [Korn et al., 1999].

From the point of view of CK2 regulation, the binding of PP2A to free CK2α suggests that when CK2β is limiting, due to its being sequestered by other kinases or its degradation, the consequence would be a decrease in CK2α kinase activity and a concomitant increase in phosphatase activity through CK2α interaction with PP2A. The relative stoichiometry of the α- and β-subunits of CK2 could therefore play a very important regulatory role that would not only control CK2 activity but one that would affect the overall balance of phosphorylation/dephosphorylation of many key proteins.

"WILD CARD" PROTEINS AS PLEIOTROPIC REGULATORS

The promiscuity of the CK2 subunits may shed some light on the factors that regulate the catalytic activity of this enzyme. In addition, the property of these subunits to act as "wild cards" pairing with several other proteins to affect the balance of whole cellular metabolic pathways merits analysis as to its generality (Fig. 2C).

Biochemists trained to expect specificity are disquieted by evidence that indicates that a protein may have a broad range of interactions. However, the further we progress in moving from the extreme reductionism of experiments in vitro with pure components towards the integration of macromolecules in physiological cellular pathways, the more we encounter the phenomenon of pleiotropy and multifunctionality of enzymes and proteins. To date, not many proteins come to mind that fit the "wild card" concept of having several other protein partners with which they can pair. Calmodulin is such a protein with many partners that become sensitive to calcium signals through its interaction. p21$^{WAF1/CIP1}$ is another example of a polypeptide that can inhibit a large number of kinases and that also binds PCNA to block DNA replication, putting a multiple brake on cell cycle progression. The subunits of trimeric G proteins also interact with several different proteins, receptors, and effectors in conveying external messages. Some other GTPases, such as Ras, could also fit the "wild card" definition. Another important example is TBP, the TATA building protein that plays many roles in widely different transcription complexes engaged with the three types of RNA polymerases. It is not surprising that these "wild card proteins" are key regulators of cellular function and are integrators or main switches that have dramatic pleiotropic effects when they are tampered with.

It would be an interesting exercise to assemble a list of these "wild card" or promiscuous proteins and even more fun to see how and when two or more of them interact with each other.

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