Protein kinase CK2: a challenge to canons

Lorenzo A. Pinna

Department of Biological Chemistry, University of Padova, V.le G. Colombo, 3 35121 Padova, Italy and Venetian Institute for Molecular Medicine (VIMM), Via Orus 2, 23129 Padova, Italy

e-mail: lorenzo.pinna@unipd.it

Journal of Cell Science 115, 3873-3878 © 2002 The Company of Biologists Ltd
doi:10.1242/jcs.00074

Summary

CK2 is an extremely conserved pleiotropic protein kinase with a growing list of more than 300 substrates, the majority of which are proteins implicated in signal transduction, gene expression and other nuclear functions. The CK2 phosphoacceptor sites are specified by multiple acidic residues, with the one at position +3 relative to the target residue being of crucial relevance. The CK2 holoenzyme is composed of two catalytic subunits (αα, α′α′ or αα′), which are essential for cell viability, and a dimer of two non-catalytic β subunits, whose precise function is still poorly understood. Although the β subunits deeply affect many properties of CK2, both the isolated catalytic subunits and the holoenzyme are constitutively active, which is probably responsible for the oncogenic potential of CK2. Given the structure of the holoenzyme, the β subunits could undergo reversible dissociation under physiological conditions and play a role as anchoring elements and/or as a docking platform for protein substrates and effectors. These unusual features are likely to be instrumental in the involvement of CK2 in a number of key biological functions, notably RNA synthesis, Wnt signaling, ubiquitination and cell survival.

Key words: Protein kinase, Casein kinase 2, Protein phosphorylation, Signal transduction, Cell regulation, Neoplasia, Apoptosis

Introduction

The increasing popularity of CK2 (an acronym derived from its former misnomer, casein kinase-2) is largely due to its terrific pleiotropy and unusual modus operandi. These are possibly two sides of the same coin, since it is hardly conceivable that an enzyme continuously at work exists at any time in a fully quiescent form – which is the case for most protein kinases, whose activities are turned on only in response to specific stimuli. In contrast with the belief that CK2 is independent of second messengers and 'spontaneously' active, in the late 1980s many reports claimed that CK2 activity can be dramatically increased in response to hormones and growth factors. However, no convincing proof was provided for the existence of the postulated 'hyperactive' forms(s) of CK2. This lack of evidence prompted me to express in 1990 the unorthodox opinion that, in the case of CK2, control by reversible phosphorylation could be implemented "in the opposite way than usually depicted: phosphorylation would represent a constitutive post-translational modification, while modulation of activity will be entirely committed to dephosphorylation" (Pinna, 1990). After Litchfield et al. re-evaluated CK2 activity in many extracts from cells treated with stimuli that had been previously reported to elicit acute increases in CK2 activity and concluded that "no reproducible increases in CK2 activity were observed in response to any of these treatments" (Litchfield, 1994), the paradigm of 'regulation equals more activity' in the case of CK2 started declining. Recent evidence indicates that the opposite (i.e. regulation equals less activity) applies in one circumstance – ensuring constitutive transcription of tRNA – when CK2 plays a central role in all eukaryotes (Ghavidel and Schultz, 2001). Meanwhile several substantial steps towards elucidation of the biological roles of CK2 have been made: many new substrates and partners have been detected; the crystal structures of its catalytic subunit (Niefind et al., 1998), of the β subunit (Chantalat et al., 1999) and of the holoenzyme (Niefind et al., 2001) have been solved, the structural features underlying many of its unique properties have been unraveled; its importance in neoplasia (Seldin and Leder, 1995; Kelliher et al., 1996) and apoptosis (Guo et al., 2000) has been demonstrated. Here I provide an updated account of this progress and speculate about the role of CK2 in some global processes.

One thousand and one substrates?

The most striking feature of CK2, responsible for its growing popularity among 'outsiders', is its pleiotropy, which is especially remarkable if one bears in mind that for two decades after its discovery in the 1950s, CK2 remained a kinase in search of its physiological substrates (Pinna, 1994). The first endogenous substrates of CK2 came to light in the early 1980s, and in 1990 50 substrates were already known. By 1994 this figure had risen to >100 and by 1997 to 160 (Pinna and Meggio, 1997). To date the repertoire of CK2 substrates includes 307 proteins (F. Meggio and L. A. Pinna, unpublished) and only in relatively few cases (50 or so) has in vitro phosphorylation not yet been corroborated by data supporting the occurrence of in vivo phosphorylation as well. This figure is steadily increasing; eventually it may include a large proportion (one tenth to one fifth) of the whole eukaryotic phosphoproteome. More telling than the number, however, is the nature of the proteins phosphorylated by CK2 and the functions they play in the cell. Not less than sixty of

the known substrates are transcription factors; these, in conjunction with >40 nuclear proteins implicated in gene expression and transcription, a dozen translational factors and >80 other proteins with various functions in signalling, make up a substantial majority of substrates implicated in the cellular signalling network. By comparison, classic metabolic enzymes, such as glycogen synthase, acetyl CoA carboxylase and ornithine decarboxylase, represent a minority (a dozen or so) of its substrates. Quite impressive instead is the list of viral proteins (38) that rely on CK2 for phosphorylation. As discussed below, this is probably a consequence of the constitutive activity of CK2, which is exploited by many viruses as a first-choice phosphorylating agent for proteins essential to their life cycle.

The fundamental roles of CK2 in signalling, gene expression and other nuclear processes have been recently highlighted in two analyses of protein complexes in yeast (Gavin et al., 2002; Ho et al., 2002), in which all four CK2 subunits (in yeast a second regulatory subunit, β′, also exists) were detected in three and four multiprotein complexes, whereas several other complexes contained two or three of the CK2 subunits. In the Gavin et al. study two or more CK2 subunits were found in seven multiprotein complexes (out of the 232 analyzed): four of these include proteins implicated in transcription, DNA maintenance or regulation of chromatin structure, one is implicated in RNA metabolism, one is implicated in protein/RNA transport and one functions in signalling. The catalytic α subunit alone is found in two additional complexes, which are involved in protein synthesis and turnover and RNA metabolism.

All known CK2 targets share typical phosphoacceptor sites specified by multiple acidic residues (on average >5) surrounding the phosphoacceptor residue, which can be serine, threonine or even, in at least one case, tyrosine. The most important acidic determinant is that at position *n*+3 and is found in nearly 90% of the sites analyzed and in all of the few sites (nine) that have only one acidic determinant. The second most important acidic determinant is that at position *n*+1 and is found in 75% of the sites. Whenever the negatively charged determinant is absent at position *n*+3, it is invariably present at position *n*+1 and vice-versa. Mutational analysis of the CK2α subunit led to the identification of a network of unique basic residues responsible for the recognition of the acidic determinants between positions *n*–1 and *n*+4 (Sarno et al., 1997). Basic residues are extremely rare at any position between *n*–1 and *n*+4 in the CK2 sites, where they behave as negative determinants. The same applies to the proline residue at position *n*+1, which conversely is part of the consensus sequence for CDKs. On the basis of these data sequences, the following motif can be held as hallmark of CK2 sites:

(E/D/x)-(S/T/y)-(D/E/x′)-(E/D/x)-(E/D)-(E/D/x)

where X is any residue except basic residues and X′ is any residue except basic or proline residues. The size of the letters is roughly proportional to the frequency of a given residue at that position. Phosphoserine (not indicated) can efficiently replace Glu and Asp at any position; the phosphoacceptor residue is underlined.

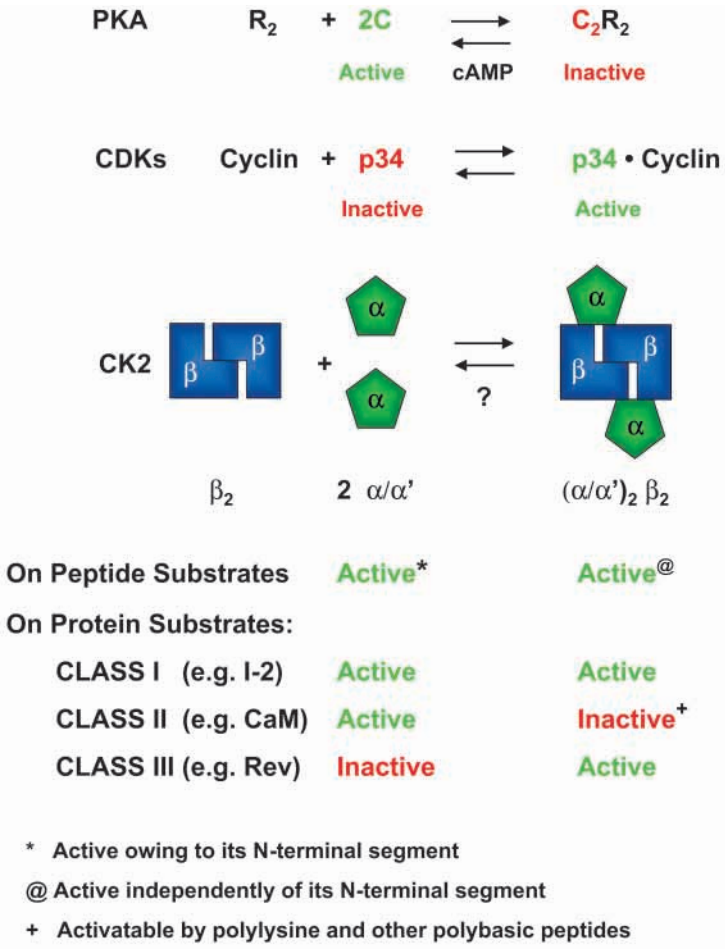

Fig. 1. The paradox of CK2 constitutive activity. Unlike PKA and CDKs, CK2 does not undergo drastic changes in catalytic activity upon association between catalytic and regulatory subunits. However the phosphorylation of some protein substrates can be deeply altered in this way.

Constitutive activity: the apparent paradox

The CK2 holoenzyme shares with few other protein kinases a quaternary structure composed of catalytic and regulatory subunits. The catalytic subunits are of two kinds, α and α′, which are encoded by distinct genes and are very similar to each other except for a C-terminal extension, present only in the α subunit, which includes proline-rich phosphoacceptor sites whose phosphorylation generates docking sites for peptidyl-prolyl isomerase Pin1 (Messenger et al., 2002). In the holoenzyme, two catalytic subunits (αα, α′α′ or αα′) associate with a dimer of the non-catalytic β subunit, which does not share homology with any other regulatory subunit of protein kinases. Quaternary structure is generally a hallmark of tight regulation. In fact the PKA holoenzyme, whose heterotetrameric structure is closely reminiscent of that of CK2, is completely inactive. Activation, promoted by cyclic AMP, involves the dissociation of the holoenzyme and the release of the free active catalytic subunits (Fig. 1). By contrast, the isolated catalytic subunits of cyclin-dependent kinases (CDKs), which belong to the same group as CK2, are inactive; in this case their heterodimeric association with a cognate cyclin is a prerequisite for activation. Full activation, however,

also requires the phosphorylation of the activation loop and can be abolished by phosphorylation of the glycine-rich loop and by association with a number of low-molecular-weight protein inhibitors. CDKs therefore must pass four checkpoints before they can become active. By contrast, CK2 is equipped with at least two independent molecular devices to keep it constitutively active: unique interactions between the activation loop and its N-terminal segment (30 residues longer than in CDKs) that confer constitutive activity upon the isolated catalytic subunit; and association of the catalytic subunits with the regulatory β subunits, which can reactivate intrinsically inactive mutants in which the contacts between the N-terminal and activation segments have been disrupted (Sarno et al., 2002). As a consequence of these redundant mechanisms of activation, both the isolated subunits and the holoenzyme are endowed with constitutive activity (Fig. 1).

The catalytic activity of the holoenzyme, determined with specific peptide substrates, is somewhat higher than that of the isolated subunits. Notable exceptions however are a limited number of protein substrates that either require the presence of the β subunit for phosphorylation or conversely are phosphorylated by the catalytic subunits but not by the holoenzyme (e.g. calmodulin). The phosphorylation of the latter group by the holoenzyme is triggered and dramatically enhanced by polybasic peptides (but not polyamines) such as polylysine. It has to be assumed that the effect of the β subunit on these particular substrates is not mediated by turning on or off catalytic activity, which is constitutively 'on', but by specific interactions with the protein substrates, perhaps in combination with polycationic stimulators.

The solution of the crystal structure of the human CK2 holoenzyme (Niefind et al., 2001) did not provide a molecular rationale for either the generic increase in catalytic activity observed in the presence of peptide substrates or the specific effect of CK2β on particular protein substrates. In the holoenzyme, a central β-β dimer holds the two catalytic subunits apart. Each subunit makes contacts with the central core of the proximal β subunit and with the C-terminal tail of the distal β subunit. These contacts do not, however, alter the overall α conformation to an extent that explains why its catalytic activity is higher than that of the isolated subunit and why it is no longer dependent on the C-terminal segment. It is also hard to figure out how the β subunit could affect accessibility to the catalytic site of protein substrates whose phosphorylation is either dependent on or prevented by the β subunit itself: the N-terminal acidic region of β, believed to account for its pseudo-substrate downregulatory potential, is far from the catalytic sites of both α subunits (Niefind et al., 2001). The crystal structure might simply provide a fragmentary view of a complex and dynamic situation in which different conformers of the CK2 holoenzyme exist in equilibrium: some of these, generated for example by the folding up of the extended, crab-shaped conformation seen in the crystal or by supramolecular association (Valero et al., 1995), might account for functional features that are otherwise hard to explain. It is also possible that the relatively low resolution of the structure (3.1 Å) has hampered the detection of subtle yet functionally relevant structural alterations that could account for changes in activity (Sarno et al., 2002).

Although the crystal structure has left unsolved a number of mechanistic issues, it has corroborated the view that the β subunit, rather than being a sensu stricto regulatory element, operates as a targeting molecule and/or a docking platform for binding substrates and effectors and for the assembly of multimolecular complexes in which CK2 plays a role. Not only does the shape of the tetramer account for the remarkable tendency of the β subunit to interact with a variety of protein 'partners' but also, and more importantly, the nature and the surface of the contacts between the β and α-subunits (832 A^2) make it quite plausible that the holoenzyme, despite its remarkable stability in vitro, "is a transient heterocomplex, which is formed and dissociates in vivo for specific functional and regulatory reasons" (Niefind et al., 2001). This revives the 'wild-card' hypothesis in which partner proteins serve as switches by variably interacting with CK2 subunits (Allende and Allende, 1998). In this respect, the β subunits of CK2 are more reminiscent of A-kinase-anchoring proteins (AKAPs) than PKA regulatory subunits (Rs), or, at least, they share some functions of both.

The lack of known physiological effectors makes the development of cell-permeable, specific inhibitors especially important for probing the cellular functions of CK2. Some widely used inhibitors are far from being selective: apigenin, for example, inhibits a dozen protein kinases more readily than it does CK2 amid a panel of 35 enzymes; and DRB (dichloro-ribofuranosyl-benzimidazole) inhibits CK1 almost as effectively as it does CK2. The most selective inhibitor described so far is tetrabromobenzotriazole (TBB) (Sarno et al., 2001), whose crystal structure in complex with CK2α (Battistutta et al., 2001) could pave the road toward the design of more potent derivatives.

Les liaisons dangeureuses: the pathogenic potential of CK2

Quite often dysregulation of protein kinases gives rise to pathological conditions and, in particular, to cancer (Blume-Jensen and Hunter, 2001). In general this is due to mutations that result in stable activation of protein kinases that otherwise are subject to tight control. In the case of CK2, constitutive activity is an intrinsic property of the enzyme itself: nevertheless it might, under certain circumstances, cause cell transformation. In fact, in contrast to other oncogenic protein kinases, there is no evidence of mutations of CK2 giving rise to tumors. However the levels of CK2 are elevated in a wide variety of tumors (reviewed by Guerra and Issinger, 1999; Tawfic et al., 2001). The cause-effect correlation between CK2 and neoplastic growth has been established by experimental models in which inappropriate expression of the CK2 α subunit in lymphocytes of transgenic mice resulted in stochastic production of lymphomas, and co-expression of the α subunit with proto-oncogenes or its expression under conditions where p53 expression was altered accelerated development of acute lymphocytic leukemia (Seldin and Leder, 1995; Kelliher et al., 1996; Landesman-Bollag et al., 1998). Also the inclusion of many viral proteins among CK2 targets strongly suggests a role for CK2 in virally mediated pathologies, including cancer. Another mechanism by which CK2 can promote dysregulated growth is inhibition of apoptosis (Guo et al., 2000). A common denominator of these observations is that modest alterations in the levels of CK2α are sufficient to induce dramatic effects on cell fate by cooperating with other factors. Dysregulation of

CK2α expression might thus impart "an oncogenic potential to the cell such that in cooperation with certain oncogenes it produces a profound enhancement of the tumor phenotype" (Tawfic et al., 2001).

Why is CK2 essential for cell life?

CK2 is one of the most conserved protein kinases in evolution, and deletion of both its catalytic subunits is lethal (Padmanabha et al., 1990). In 1990, terming CK2 *an eminence grise*, I wanted to stress the fact that it acts behind the scenes in such a way that it is indispensable, although it does not appear to play any prominent role in the hierarchy of cellular functions. A hurried statement would be that CK2 is of vital importance because of its pleiotropism: it is in charge of so many tasks that, although individually none might be indispensable, they cannot be collectively omitted without fatal consequences. It would be nice, however, to show that behind its apparent randomness CK2 performs a number of global tasks within the cell and that there are specific means of regulating its activity at least within a given process. There are some emerging clues that indicate that this is the case.

The PolIII connection

The RNA polymerase I and RNA polymerase II complexes were among the first CK2 substrates to be discovered in the early eighties. It was not until 1996 that the RNA polymerase III (PolIII) machinery was also shown to be a target of CK2 and to belong to the 'élite' whose phosphorylation by CK2 obviously correlates with a change in biological activity. Phosphorylation by CK2 of the TATA-binding protein (TBP), a subunit of TFIIIB (the core component of the PolIII transcriptional machinery), promotes a remarkable increase in PolIII activity, although the step in initiation stimulated by CK2 is not known. The PolIII model is especially appealing because, being committed to the synthesis of tRNA and 5SrRNA, PolIII is expected to be constantly active in living cells except under special circumstances in which transcription has to be interrupted. Typically these breaks in PolIII activity are imposed by a signaling pathway generated in response to DNA damage. Gavidel and Schultz have shown that CK2 normally associates through its β subunit with the TBP subunit of TFIIIB and by doing that phosphorylates TBP and sustains PolIII transcription (Gavidel and Schultz, 2001). Indeed disruption of CK2 causes the synthesis of tRNA and 5SrRNA to decline by 80-90%. Transcriptional repression induced by DNA damage is mediated by downregulation of TBP-associated CK2, which occurs through the release of the catalytic subunits from the complex. These data support the view that CK2 is the terminal effector in a signaling pathway that represses PolIII transcription when genome integrity is compromised and suggest that the molecular mechanism leading to downregulation of CK2 is the dissociation of the catalytic subunits from the β-β dimer anchored to the complex – an event plausible in the context of the crystallographic data discussed above. This would imply that changes in conformation weaken the interactions between the TBP-associated β-β dimer and the catalytic subunits, which would be expelled from the complex. How this might take place and how the signals from DNA damage sensors are transduced to TBP-bound CK2 are unknown. In any case, a remarkable merit of this study is that it highlights a central function of CK2 in which its two paradoxical properties, constitutive activity and regulation by deactivation, not only make sense but are instrumental to the whole process.

The Wnt pathway connection

Wnt signalling, initiated by secreted Wnt proteins that bind to members of the Frizzled receptor family, plays a central role in development and homeostasis. A key element in the pathway is the level of cytosolic β-catenin, which triggers the activation of Wnt responsive genes. Without Wnt stimulation, β-catenin is steadily degraded by the proteasome. This degradation strictly depends upon β-catenin phosphorylation occurring in a multiprotein complex by a hierarchical mechanism involving both CK1 and glycogen synthase kinase 3 (GSK3). This phosphorylation is critical for binding of β-catenin to the F-box protein β-TrCP, which imparts specificity on the ubiquitination apparatus. Wnt signaling inhibits GSK3 and prevents the ubiquitination and degradation of β-catenin, thus inducing its accumulation in the cytosol and the activation of Wnt responsive genes, some of which are proto-oncogenes. Dysregulated activation of the Wnt signal can therefore give rise to tumors, and inhibition of GSK3, a potential target for diabetes therapy, in principle could cause cancer. A first indication that CK2 is also implicated in Wnt signalling was provided by Willert et al. who showed that CK2 associates with and phosphorylates Dsh (Willert et al., 1997), the *Drosophila* homolog of mammalian Dvl, which is a key component of the regulatory complex in which the GSK3-catalyzed phosphorylation of β-catenin is abrogated by Frat-1. More recently Song et al. have shown that increased proliferation of a mammary epithelial cell line after Wnt1 transfection is accompanied by increased levels of CK2α and β-catenin (Song et al., 2000). They also showed that CK2 forms complexes with β-catenin and Dvl, which do not, however, include GSK3. Inhibition of CK2 reduces the level of β-catenin and blocks proliferation of Wnt-transfected cells, suggesting that β-catenin phosphorylated by CK2 escapes ubiquitination and degradation. Consequently when GSK3 is inhibited by Wnt signalling, phosphorylation of β-catenin by CK2 would become predominant, leading to an increased level of β-catenin available to activate Wnt-responsive genes. Although the residue(s) of β-catenin phosphorylated by CK2 are not known, these are obviously different from those affected by GSK3. The work of Song et al. thus provides a clue to the oncogenic potential of CK2 (Song et al., 2000), inasmuch as β-catenin is also upregulated in transgenic tumors induced by CK2α.

Note, however, that both β-catenin and CK2 play more intricate and apparently controversial roles in the cell than it might appear from this straightforward model. Under different experimental conditions, β-catenin overexpression blocks the cell cycle and induces apoptosis instead of proliferation (Kim et al., 2000), and in fibroblasts, CK2 phosphorylates UBC3 and by doing so activates the F-box of the ubiquitination machinery, ultimately promoting the degradation of cytosolic β-catenin (Semplici et al., 2002). Once again the pleiotropy of CK2 hampers an unequivocal explanation of its functions. It is tempting to speculate that in this case the dissociation of CK2 holoenzyme switches its action from one pathway to another

(Fig. 2). Although phosphorylation of UBC3 is strongly stimulated in vitro and perhaps entirely dependent in vivo on the β-subunit (Semplici et al., 2002), phosphorylation of β-catenin could rely on the catalytic subunit alone. Two observations are consistent with this possibility: the lack of CK2β in immunoprecipitates in which CK2α and β-catenin are present together; and the finding that β-catenin is upregulated in transgenic tumors tranfected with CK2α alone (Song et al., 2000). Such a scenario is also consistent with the view that the oncogenic potential of CK2α is not enhanced but eventually decreased by co-transfection of the β-subunit (Li et al., 1999) and that increased expression of CK2β results in increases in total cellular CK2 activity but no changes in cell proliferation (Vilk et al., 2002). Moreover, the existence of two pools of CK2, both constitutively active but having different functions, would account for the redundancy of CK2 activation mechanisms, ensuring that not only the holoenzyme but also the isolated catalytic subunits are constitutively active (Sarno et al., 2002).

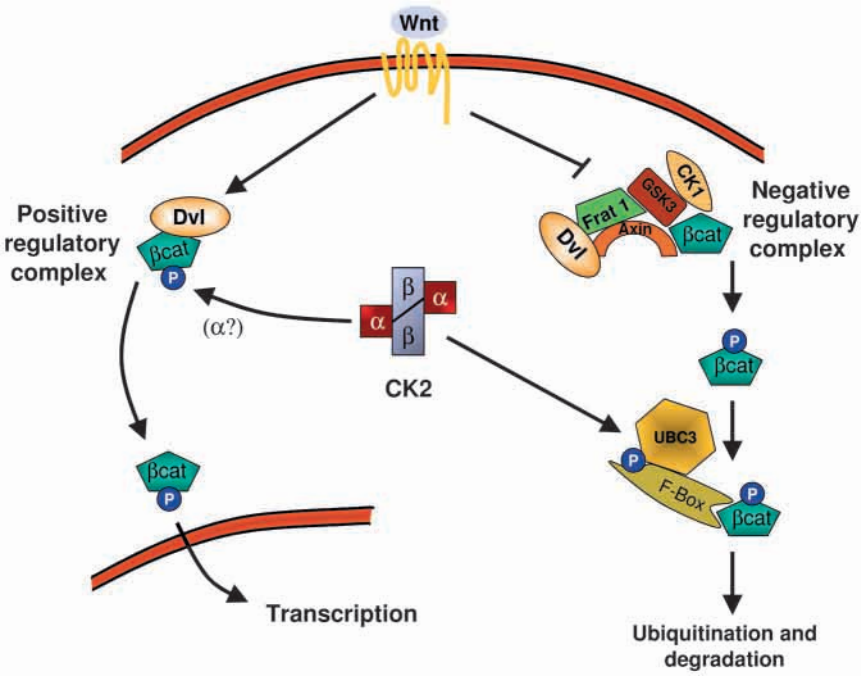

Fig. 2. Hypothesized dual role of CK2 in the Wnt signaling pathway. Direct phosphorylation by CK2 prevents β-catenin degradation, counteracting the effect of GSK3 phosphorylation within the negative regulatory complex (Son et al., 2000). On the other hand, CK2, by phosphorylating the E2 ubiquitin-conjugating enzyme UBC3, promotes its binding to the proteasome receptor β-TrCP, ultimately assisting the degradation of β-catenin (Semplici et al., 2002).

The caspase connection

Ruzzene et al. have recently provided evidence to support the concept that CK2 counteracts apoptosis by showing that a specific CK2 inhibitor (TBB) induces apoptosis in Jurkat cells (Ruzzene et al., 2002). In the course of this study they observed that inhibition of CK2 promoted the dephosphorylation of the apoptotic protein HS1 and its accelerated degradation by caspases. They also showed that in vitro CK2-mediated phosphorylation prevents the cleavage of HS1 by caspase 3. Interestingly HS1 is not the only protein made refractory to caspase cleavage by CK2 phosphorylation: phosphorylation of Max (Krippner-Heidenreich et al., 2001), Bid (Desagher et al., 2001), connexin 45.6 (Yin et al., 2001) and probably also presenilin-2 (Walter et al., 1999) by CK2 also downregulates caspase-dependent degradation. Whether the adverse effect of CK2 on caspase cleavage is site directed (as suggested, in some cases, by the proximity of the phosphorylated residue to the site of cleavage) or mediated by conformational changes, is still an open question. Nevertheless this observation opens a window on a possible general mechanism by which CK2 counteracts apoptosis and provides a unifying theory to explain the phosphorylation by CK2 of otherwise unrelated sets of proteins.

Conclusions and perspectives

All data available indicate that CK2 is the most pleiotropic of all protein kinases, with hundreds of substrates implicated in a wide variety of cellular functions. The data also support the view that CK2 plays a global role in cell regulation and has particularly important functions in gene expression, signal transduction and cell survival. Evidence is accumulating that the paradoxical properties of CK2, notably its high constitutive activity and lack of an acute mode of regulation, are instrumental in its extreme pleiotropy. In this respect, CK2 cannot be compared with any other protein kinase. It should be considered to be an independent executor whose interplay with the network of signaling pathways is genetically predisposed but may be not readily adjustable. CK2 is clearly implicated in dysregulated cell proliferation and virus infection, although the molecular mechanisms involved are not yet precisely understood. The most important question now is whether the formation of CK2 holoenzyme is a reversible process inside the cell. If so, this would account for many in vivo observations and could reconcile some controversial data. Another important question is whether the dramatic stimulation of CK2 holoenzyme by basic polypeptides, which are responsible for all-or-none effect toward certain substrates, is an in vitro artifact or reflects an in vivo reality, possibly mediated by polycationic domains present in many CK2 substrates and partners. Both questions converge into one: what is the role of the β subunit? Is it a canonical regulator or a targeting protein, a scaffold, a docking subunit or, as seems likely, all of these? Important insights into this issue will come from knock-out animals lacking the β subunit, from a more detailed and dynamic scrutiny of the structure of the CK2 holoenzyme and from the solution of the crystal structure of complexes of the holoenzyme and phosphoacceptor substrates whose phosphorylation is variably affected by the β subunit. No doubt in the future we will see a further extension of the already long list of CK2 substrates and partners. Some of these may shed new light on the biological functions of CK2, but I am afraid

that altogether they will only make the scenario even more complex until the basal questions regarding the reversibility of holoenzyme formation and the functions of the β subunit have been answered. A key goal is the development of more potent and selective inhibitors of CK2 that are ideally capable of discriminating between the catalytic subunits and the holoenzyme. These reagents should help us dissect the cellular functions of CK2 and make studies less dependent on transfection experiments, which, no matter how ingenious, are often inconclusive and sometimes misleading.

I am grateful to many colleagues for helpful advice and suggestions. Special thanks are due to Khalil Ahmed, Claiborne V.C. Glover, Mike Schultz and David Seldin for enlightening comments, to Flavio Meggio for updating the repertoire of CK2 substrates and to Carol Ponzetto for linguistic advice. The technical aid of Luca Cesaro is also acknowledged.

References

Allende, C. C. and Allende, J. E. (1999). Promiscuous subunit interactions: a possible mechanism for the regulation of protein kinase CK2. *J. Cell. Biochem.* **30**, 129-136.

Battistutta, R., DeMoliner, E., Sarno, S., Zanotti, G. and Pinna, L. A. (2001). Structural features underlying the selective inhibition of protein kinase CK2 by ATP site-directed tetrabromo-2-benzotriazole. *Protein Sci.* **10**, 2200-2206.

Blume-Jensen, P. and Hunter, T. (2001). Oncogenic kinase signalling. *Nature* **411**, 355-365.

Chantalat, L., Leroy, D., Filhol, O., Nueda, A., Benitez, M. J., Chambaz, R. M., Cochet, C. and Dideberg, O. (1999). Crystal structure of the human protein kinase CK2 regulatory subunit reveals its zinc finger mediated dimerization. *EMBO J.***18**, 2930-2940.

Desagher, S., Osen-Sand, A., Montessuit, S., Magnenat, E., Vilbois, F., Hochmann, A., Journot, L., Antonsson, B. and Martinou, J. C. (2001). Phosphorylation of Bid by casein kinase I and II regulates its cleavage by caspase 8. *Mol. Cell* **8**, 601-611.

Gavin, A.-C. et al. (2002). Functional organization of the yeast proteome by systematic analysis of protein complexes *Nature* **415**, 141-147.

Ghavidel, A. and Schultz, M. C. (2001). TATA binding protein-associated CK2 transduces DNA damage signals to the RNA polymerase III transcription machinery. *Cell* **106**, 575-584.

Guerra, B. and Issinger, O.-G. (1999). Protein kinase CK2 and its role in cellular proliferation, development and pathology *Electrophoresis* **20**, 391-408.

Guo, C., Yu, S., Wang, H., Davis, A. T., Green, J. E. and Ahmed, K. (2000). A potential role of nucler matrix-associated protein kinase CK2 in protection against drug-induced apoptosis in cancer cells *J. Biol. Chem.* **276**, 5992-5999.

Ho, Y., Gruhler, A., Heilbut, A., Bader, G. D., Moore, L., Adams, S.-L., Millar, A., Taylor, P., Bennet, K., Boutilier, K. et al. (2002). Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry. *Nature* **415**, 180-183.

Kelliher, M. A., Seldin, D. and Leder, P. (1996). Tal-1 induces T cell acute lymphoblastic leukemia accelerated by casein kinase IIα. *EMBO J.* **15**, 5160-5166.

Kim, K., Pand, K. M., Evans, M. and Hay, E. D. (2000). Overexpression of beta-catenin induces apoptosis independent of its transactivation function with LEF-1 or the involvement of major G1 cell cycle regulators. *Mol. Biol. Cell* **11**, 3509-3523.

Krippner-Heidenreich, A., Talanian, R. V., Sekul, R., Kraft, R., Thole, H., Ottleben, H. and Luscher, B. (2001) Trageting of the transcription factor Max during apoptosis: phosphorylation regulated cleavage by caspase-5 at an unusual glutamic acid at position P1. *Biochem. J.* **358**, 705-715.

Lansedman-Bollag, E., Channavajhala, P. L., Cardiff, R. D. and Seldin, D. C. (1998). p53 deficiency and misexpression of protein kinase CK2 alpha collaborate in the development of thymic lymphomas in mice. *Oncogene* **16**, 2965-2974.

Li, D., Dobrowolska, G., Aicher, L. D., Chen, M., Wright, J. H., Drueckes, P., Dunphy, E. L., Munar, E. S. and Krebs, E. G. (1999) Expression of the casein kinase 2 subunits in Chinese hamster ovary and 3T3 L1 cells provides information on the role of the enzyme in cell proliferation and the cell cycle. *J. Biol. Chem.* **274**, 32988-32996.

Litchfield, D. W., Dobrowolska, G. and Krebs, E. G. (1994) Regulation of casein kinase II by growth factors: a reevaluation *Cell. Mol. Biol. Res.* **40**, 373-381.

Messenger, M. M., Saulnier, R. B., Gilchrist, A. D., Diamond, P., Gorbsky, G. J. and Litchfield, D. W. (2002). Interactions between protein kinase CK2 and Pin1: evidence for phosphorylation-dependent interactions. *J. Biol. Chem.* **277**, 23054-23067.

Niefind, K., Guerra, B., Ermakowa, I. and Issinger, O.-G. (2001). Crystal structure of humen protein kinase CK2: insights into basic properties of the CK2 holoenzyme. *EMBO J.* **20**, 5320-5331.

Niefind, K., Guerra, B., Pinna, L. A. and Issinger, O.-G. (1998). Crystal structure of the catalytic subunit of protein kinase CK2 from *Zea mays* at 2.1 Å resolution *EMBO J.* **17**, 2451-2462.

Padmanabha, R., Chen-Wu, J. L.-P., Hanna, D. E. and Glover, C. V. C. (1990). 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.

Pinna, L. A. (1990). Casein kinase 2: an “eminence grise” in cellular regulation? *Biochim. Biophys Acta* **1054**, 267-284.

Pinna, L. A. (1994). A historical view of protein kinase CK2. *Cell. Mol. Biol. Res.* **40**, 383-390.

Pinna, L. A. and Meggio, F. (1997). Protein kinase CK2 (“casein kinase-2”) and its implication in cell division and proliferation. In *Progress in Cell Cycle Research Vol. 3* (eds L. Meijer, S. Guidet and M. Philippe), pp. 77-97. New York, USA: Plenum Press.

Ruzzene, M., Penzo, D. and Pinna, L. A. (2002). Protein kinase CK2 inhibitor 4,5,6,7-tetrabromobenzotriazole (TBB) induces apoptosis and caspase dependent degradation of haematopoietic lineage cell specific protein 1 (HS1) in Jurkat cells. *Biochem. J.* **364**, 41-47.

Sarno, S., Vaglio, P., Marin, O., Issinger, O.-G., Ruffato, K. and Pinna, L. A. (1997). Mutational analysis of residues implicated in the interaction between protein kinase CK2 and peptide substrates. *Biochemistry* **36**, 11717-11724.

Sarno, S., Reddy, H., Meggio, F., Davies, S. P., Donella-Deana, A., Shugar, D. and Pinna, L. A. (2001). Selectivity of 4,5,6,7-tertrabromobenzotriazole, and ATP site-directed inhibitor of protein kinase CK2. *FEBS Lett.* **496**, 44-48.

Sarno, S., Ghisellini, P. and Pinna, L. A. (2002). Unique activation mechanism of protein kinase CK2: the N-terminal segment is essential for constitutive activity of the catalytic subunit but not of the holoenzyme. *J. Biol. Chem.* **277**, 22509-22514.

Seldin, D. and Leder, P. (1995). Casein kinase IIα transgene-induced murine lymphoma: relation to theileriosis in cattle. *Science* **267**, 894-897.

Semplici, F., Meggio, F., Pinna, L. A. and Oliviero, S. (2002). CK2 dependent phosphorylation of the E2 ubiquitin conjugating enzyme UBC3B induces its interaction with β-TrCP and enhances β-catenin degradation. *Oncogene* **21**, 3978-3987.

Song, D. H., Sussman, D. J. and Seldin, D. C. (2000). Endogenous protein kinase CK2 participates in Wnt signaling in mammary epithelial cells. *J. Biol. Chem.* **275**, 23790-23797.

Tawfic, S., Yu, S., Wang, H., Faust, R., Davis, A. and Ahmed, K. (2001). Protein kinase CK2 signal in neoplasia. *Histol. Histopathol.* **16**, 573-582.

Valero, E., de Bonis, S., Filhol, O., Wade, R. H., Langowski, J., Chambaz, E. M. and Cochet, C. (1995) Quaternary structure of protein kinase CK2. Characterization of multiple oligomeric states and relation with its catalytic activity. *J. Biol. Chem.* **270**, 8345-8352.

Vilk, G., Derksen, D. R. and Litchfield, D. W. (2002). Inducible expression of the regulatory protein kinase CK2β subunit: incorporation into complexes with catalytic CK2 subunits and re-examination of the effects of CK2β on cell proliferation. *J. Cell. Biochem.* **84**, 84-99.

Walter, J., Schindzielorz, A., Gruenberg, J. and Haass, C. (1999). Phosphorylation of presenilin-2 regulates its cleavage by caspases and retards progression of apoptosis. *Proc. Natl. Acad. Sci. USA* **96**, 1391-1396.

Willert, K., Brink, M., Wodarz, A., Varmus, H. and Nusse, R. (1997). Casein kinase 2 associates with and phosphorylates dishevelled. *EMBO J.* **16**, 3089-3096.

Yin, X., Gu, S. and Jiang, J. X. (2001). The development-associated cleavage of lens connexin 45.6 by caspase-3-like protease is regulated by casein kinase II-mediated phosphorylation. *J. Biol. Chem.* **276**, 34567-34572.