REVIEW ARTICLE

Protein kinase CK2: structure, regulation and role in cellular decisions of life and death

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Protein kinase CK2 ('casein kinase II') has traditionally been classified as a messenger-independent protein serine/threonine kinase that is typically found in tetrameric complexes consisting of two catalytic (α and/or α') subunits and two regulatory β subunits. Accumulated biochemical and genetic evidence indicates that CK2 has a vast array of candidate physiological targets and participates in a complex series of cellular functions, including the maintenance of cell viability. This review summarizes current knowledge of the structural and enzymic features of CK2, and discusses advances that challenge traditional views of this enzyme. For example, the recent demonstrations that individual CK2 subunits exist outside tetrameric complexes and that CK2 displays dual-specificity kinase activity raises new prospects for the precise elucidation of its regulation and cellular functions. This review also discusses a number of the mechanisms that contribute to the regulation of CK2 in cells, and will highlight emerging insights into the role of CK2 in cellular decisions of life and death. In this latter respect, recent evidence suggests that CK2 can exert an anti-apoptotic role by protecting regulatory proteins from caspase-mediated degradation. The mechanistic basis of the observation that CK2 is essential for viability may reside in part in this ability to protect cellular proteins from caspase action. Furthermore, this anti-apoptotic function of CK2 may contribute to its ability to participate in transformation and tumorigenesis.

Key words: apoptosis, cell cycle, cell survival, dual-specificity kinase, phosphorylation.

INTRODUCTION

For many years, it has been abundantly clear that the reversible phosphorylation of proteins is a major mechanism for the regulation of a broad spectrum of fundamental cellular processes [1,2]. Given the importance of this covalent modification, it may not be surprising that the human genome encodes several hundred distinct protein kinases [3,4], that a third of all cellular proteins appear to be phosphorylated [5], and that many proteins are phosphorylated at several distinct sites [6]. Accordingly, on average, each protein kinase may phosphorylate a few dozen proteins within a cell. However, it is apparent that some protein kinases, such as wee1 and MEK [mitogen-activated protein (MAP) kinase/extracellular-signal-regulated kinase (ERK) kinase], are exquisitely specific and are present in cells to phosphorylate perhaps only one or two distinct protein targets. By comparison, many other protein kinases exhibit a much broader specificity and are likely to phosphorylate hundreds of distinct proteins within cells. Protein kinase CK2 (an acronym derived from the misnomer 'casein kinase II') represents a small family of closely related protein kinases that clearly falls into the latter category [7–15]. Ironically, although well over 100 potential physiological targets of CK2 have been identified to date, it seems very unlikely that CK2 has any role in the *in vivo* phosphorylation of casein, the protein from which it originally derived its name [16].

As the list of likely physiological targets for CK2 continues to grow, it becomes increasingly evident that CK2 has the potential to participate in the regulation of a diverse selection of cellular processes. Consequently, a comprehensive compilation of all of the cellular targets of CK2 and a description of all of its reported cellular functions is beyond the scope of the present article. Instead, this review will summarize recent advances in our understanding of the structural, enzymic and regulatory features of the CK2 family of enzymes, and the involvement of CK2 in cellular decisions of life and death.

GENERAL STRUCTURAL AND ENZYMIC FEATURES OF CK2

Protein kinase CK2 is distributed ubiquitously in eukaryotic organisms, where it most often appears to exist in tetrameric complexes consisting of two catalytic subunits and two regulatory subunits (Figure 1). In many organisms, distinct isoenzymic forms of the catalytic subunit of CK2 have been identified [17–21]. For example, in humans, two catalytic isoforms, designated CK2α and CK2α', have been well characterized, while a third isoform, designated CK2α'', has been identified recently [22,23]. In humans, only a single regulatory subunit, designated CK2β, has been identified, but multiple forms of CK2β have been identified in other organisms, such as *Saccharomyces cerevisiae* [20]. Several complementary lines of evidence indicate that dimers of CK2β are at the core of the tetrameric CK2 complexes [14, 24–29]. With mammalian CK2, tetrameric CK2 complexes may contain identical (i.e. two CK2α or two CK2α') or non-identical (i.e. one CK2α and one CK2α') catalytic subunits [24].

At a very early stage after its discovery, CK2, together with a distinct casein kinase designated 'casein kinase I' (now known as protein kinase CK1), was distinguished among known protein kinases for its ability to phosphorylate serine or threonine residues that are proximal to acidic amino acids [7]. Systematic

Abbreviations used: ARC, apoptosis repressor with caspase recruitment domain; CK2, protein kinase CK2 ('casein kinase II'); CKIP-1, CK2-interacting protein-1; FAF, Fas-associated factor; FGF, fibroblast growth factor; HS1, haematopoietic lineage cell-specific protein 1; MAP kinase, mitogen-activated protein kinase; PP2A, protein phosphatase 2A; TBB, 4,5,6,7-tetrabromobenzotriazole; TNF-α, tumour necrosis factor-α.

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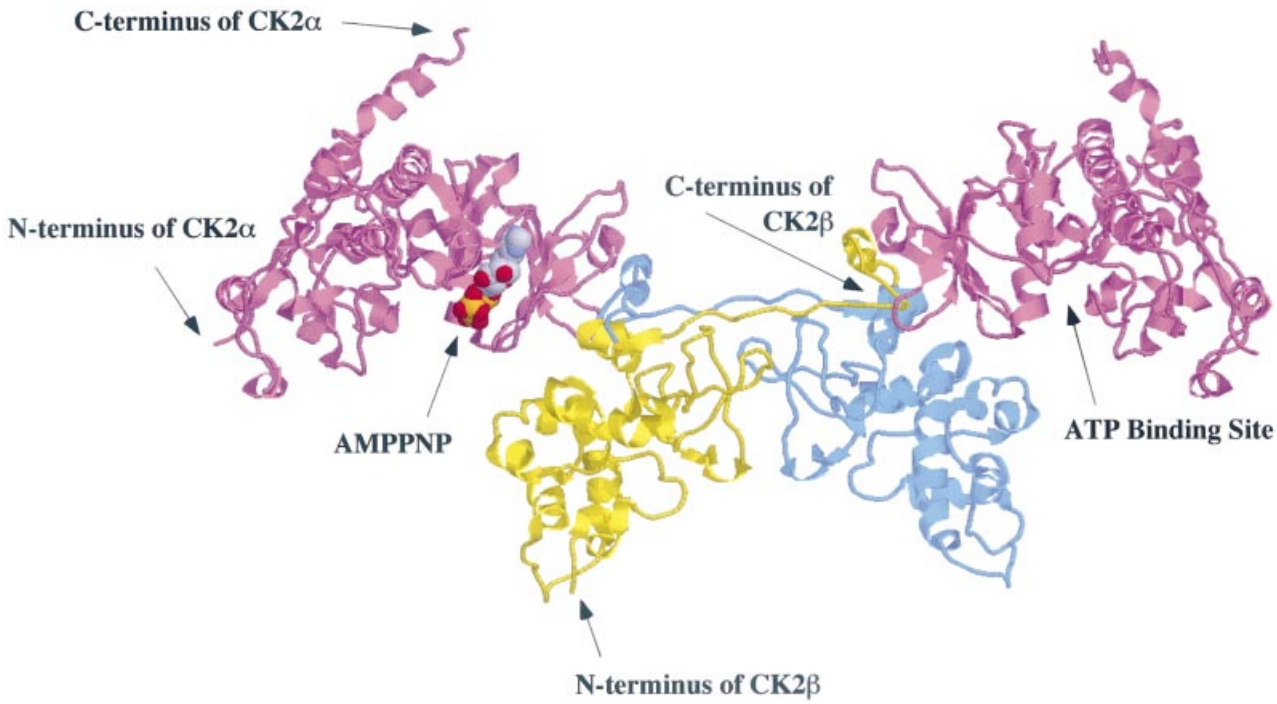

Figure 1 Ribbon diagram illustrating the high-resolution structure of tetrameric CK2

The co-ordinates ([205]; PDB identification number IJWH) from the high-resolution crystal structure of the CK2 holoenzyme [29] were used with RASMOL [206] to generate a ribbon diagram illustrating the CK2 tetramer. The catalytic CK2α subunits are illustrated in magenta. One regulatory CK2β subunit is illustrated in yellow and the other CK2β subunit is illustrated in blue. The N-termini and C-termini for one CK2α subunit and for the CK2β subunit that is illustrated in yellow are indicated. The high-resolution structure of tetrameric CK2 demonstrated that the non-hydrolysable ATP analogue adenosine 5′-[β,γ-imido]triphosphate (AMPPNP) is present in the ATP binding site of only one of the catalytic CK2α subunits (i.e. the one shown on the left) within the CK2 tetramer. The significance of this observation remains unknown.

studies, particularly those performed by Pinna and colleagues, subsequently led to the definition of a minimal consensus sequence for phosphorylation by CK2 (i.e. Ser-Xaa-Xaa-Acidic, where the acidic residue may be Glu, Asp, pSer or pTyr) that remains distinct from the minimal consensus sequence for any other protein kinase that has been characterized to date [7,14]. While delineation of this minimal consensus sequence has greatly facilitated the identification of many new potential CK2 targets, there are limitations to the use of such a consensus sequence for the identification of such targets. For example, there are sites, such as Ser^{392} in the p53 tumour suppressor, that are efficiently phosphorylated by CK2 despite the fact that they do not conform to this consensus sequence [30]. Conversely, the presence of a minimal consensus sequence for CK2-mediated phosphorylation does not guarantee efficient phosphorylation, since there may be additional determinants within the sequence that modulate phosphorylation efficiency [31].

CK2 AS A DUAL-SPECIFICITY KINASE

On the basis of its amino acid sequence and initial biochemical characterization, CK2 has traditionally been classified as a protein serine/threonine kinase [32,33]. While there is ample evidence demonstrating that CK2 can effectively phosphorylate serine or threonine residues, it has become apparent that CK2 may in fact be a dual-specificity kinase. For example, in yeast, which lack *bona fide* protein tyrosine kinases, the yeast nucleolar immunophilin FPR3 is phosphorylated at Tyr^{184} in a CK2-dependent manner (i.e. Tyr^{184} is not phosphorylated at the non-permissive temperature in yeast with a temperature-sensitive allele of CK2) [34]. The ability of CK2 to phosphorylate this residue *in vitro* is consistent with the conclusion that Tyr^{184} is phosphorylated directly by CK2 in yeast and that CK2 is a dual-specificity kinase, at least in *S. cerevisiae*. The ability of CK2 to phosphorylate tyrosine residues *in vitro* was confirmed with yeast CK2 and with recombinant human CK2 [35–37]. However, a systematic investigation of the CK2-catalysed phosphorylation of synthetic peptides containing phosphorylatable serine, threonine or tyrosine residues indicated that the kinetic parameters for tyrosine-containing peptides are much less favourable than those for serine-containing peptides [35]. Despite the modest tyrosine phosphorylation that is observed *in vitro*, it is conceivable that CK2 may also be a dual-specificity kinase in mammalian cells, as it appears to be in yeast. Since the specificity determinants for tyrosine phosphorylation by CK2 may differ from those seen for serine or threonine phosphorylation [35], the prospect that CK2 can function as a dual-specificity kinase in living cells further expands the possibilities for the identification of its substrates and elucidation of its cellular functions.

CATALYTIC SUBUNITS OF CK2: FUNCTIONAL COMPENSATION AND SPECIALIZATION

To date, much of the literature involving CK2 has not made a distinction between the different isoenzymic forms of CK2. In particular, given the close similarity in the enzymic characteristics of CK2α and CK2α' (and presumably CK2α''), it is not possible from simple CK2 phosphorylation assays to determine which isoforms are actually contributing to the activity under investigation [38]. Although closely related, CK2α and CK2α' are in

fact the products of different genes [39,40]. In their catalytic domains, CK2α and CK2α' exhibit approx. 90% identity (reviewed in [13]), providing a rational explanation for the fact that they display similar enzymic properties (including turnover rates and substrate specificity) *in vitro* [38]. In contrast with the high similarity that is seen within their catalytic domains, the C-terminal domains of CK2α and CK2α' are completely unrelated (reviewed in [13]). Notably, the unique C-termini of CK2α and CK2α' are highly conserved between species, suggesting that important functional features may be encoded within these domains. For example, the amino acid sequences of human and chicken CK2α exhibit 98% identity, while the CK2α' sequences exhibit 97% identity between these species [17,19]. Very little is currently known about CK2α'', which was identified only recently [23]. On the basis of its amino acid sequence, CK2α'' is most closely related to CK2α [23]. In fact, with the exception of position 127 (Thr in CK2α and Ala in CK2α''), the first 353 amino acids of CK2α and CK2α'' are identical. By comparison, the C-terminal 32 amino acids of CK2α'' are completely unrelated to the C-terminal 37 amino acids of CK2α. Since information regarding CK2α'' remains limited, the following discussion will emphasize functional differences between CK2α and CK2α'.

While knockouts of CK2α (or CK2α'') have not yet been reported, a knockout of the gene encoding CK2α' in mice results in viable offspring when heterozygous mice are bred to homozygosity, suggesting that CK2α (or perhaps CK2α'') has the capacity to compensate for CK2α' in the context of viability [41]. However, the male offspring are sterile and display a defect in spermatogenesis, demonstrating that the ability of CK2α to compensate functionally for the lack of CK2α' is not absolute. These results are analogous with the situation in the yeast *S. cerevisiae*, which also harbours two catalytic CK2 isoenzymes, designated CKA1 and CKA2 [20,42,43]. Yeast with a disruption of either CKA1 or CKA2 remain viable, while disruption of both CKA1 and CKA2 is synthetic lethal. As was the case in mice, this result indicates that the two isoforms of CK2 can compensate for each other in the context of viability. However, as in mice, functional overlap between CKA1 and CKA2 is incomplete, since yeast with temperature-sensitive alleles of CKA1 or CKA2 exhibit distinct phenotypes [20].

In mammalian cells, there is additional evidence for functional distinctions between CK2α and CK2α'. For example, CK2α is phosphorylated at sites within its unique C-terminal domain in a cell cycle-dependent manner, implying that CK2α and CK2α' are differentially regulated during the cell cycle [44,45]. Characterization of human osteosarcoma U2-OS cell lines that were stably transfected to achieve tetracycline-regulated expression of catalytically inactive forms of CK2α or CK2α' provides further evidence for functional specialization of CK2α and CK2α' at the cellular level [46]. In these cells, induced expression of catalytically inactive CK2α' resulted in a significant decrease in proliferation, while similar effects on proliferation were not observed upon induction of even higher levels of catalytically inactive CK2α. There may also be differences in the subcellular localization of CK2α and CK2α', although this has been an area of debate within the field [8,47–51]. Although discrepancies regarding the precise localization of the individual CK2 isoforms remain, it is apparent that CK2α and CK2α' are not functionally identical in yeast or in mammals.

Further support for the existence of functional differences in CK2α and CK2α' in mammalian cells comes from the identification of isoform-specific interacting proteins. In mammalian cells, the serine/threonine phosphatase PP2A (protein phosphatase 2A) was the first isoform-specific interaction partner to be identified for CK2 [52]. PP2A has been shown to bind to CK2α in a manner that is dependent on a sequence in CK2α (HEHRKL) that closely resembles the PP2A binding site of the simian virus 40 small t antigen (HENRKL). Although the possibility that PP2A binds to CK2α' has not been rigorously excluded, the corresponding sequence of CK2α' (HQQKKL) is distinct from those of CK2α and the small t antigen. In an effort to begin to understand the mechanistic basis for the functional specialization of CK2α and CK2α', a systematic study was performed using the yeast two-hybrid system to identify CK2α- or CK2α'-interacting proteins [22,53]. These studies yielded a novel CK2-interacting protein (designated CKIP-1) that interacts with CK2α, but not with CK2α'. A third CK2α-specific interacting protein is the peptidyl-prolyl isomerase Pin1, a recently identified protein with important functions associated with a variety of cellular processes, including cell division [54,55]. Interactions between CK2α and CKIP-1 or Pin1 will be discussed in the context of CK2 regulation later in this review.

Given the complex nature of CK2, in terms of its large number of potential substrates and its participation in a broad array of cellular processes, it is inevitable that many more isoform-specific functions or interactions for each of the CK2 isoforms remain to be defined. In fact, systematic studies that have been performed on a global scale in yeast utilizing the two-hybrid system or by mass spectrometric analysis of affinity-purified protein complexes provide further indications that the two forms of yeast CK2 (CKA1 and CKA2) do not exhibit a completely overlapping series of interactions [56–58].

THE REGULATORY CK2β SUBUNIT: ITS ROLE AS A CK2 SUBUNIT

Remarkably, the amino acid sequence of CK2β is even more highly conserved between species than is the amino acid sequence of either of the catalytic subunits. In fact, its entire 215-amino-acid sequence is identical between birds and mammals, with these sequences differing from that of *Xenopus laevis* by only a single conservative amino acid substitution [19,59–62]. As illustrated in Figure 2, CK2β has a number of characteristic features. For example, CK2β contains an autophosphorylation site comprising Ser^2, Ser^3 and possibly Ser^4 at its N-terminus [38,63,64]. Based on kinetic measurements, autophosphorylation of CK2 was originally classified as an intramolecular process [65]. However, the recent high-resolution structure of tetrameric CK2 that was determined by X-ray crystallography raises questions regarding the precise mechanism of autophosphorylation, since the N-terminus of CK2β is located at a distance of more than 40 Å (Å ≡ 0.1 nm) from the active site of either of the catalytic subunits within the tetrameric CK2 complex [29] (Figure 1). One possible mechanism for autophosphorylation may reside in the formation of higher-order CK2 structures between CK2 tetramers that have been characterized *in vitro* [66,67]. Although the physiological relevance of higher-order CK2 structures remains unclear, it is evident that a major proportion of CK2β is phosphorylated at its autophosphorylation site in cells [64,68]. CK2β is also phosphorylated at Ser^{209}, a site that is phosphorylated in a cell cycle-dependent manner in cells and *in vitro* by $p34^{cdc2}$ [64,69,70]. A more thorough discussion of the potential role of these phosphorylation sites in the regulation of CK2 will appear later in this review.

In addition to its phosphorylation sites, CK2β has a number of additional features that are noteworthy (Figure 2). Initially noted by Allende and Allende [12], CK2β contains a sequence that very closely resembles the destruction box that confers mitosis-specific degradation to cyclin B [71–74]. While it does not appear that the putative destruction box of CK2β acts alone to

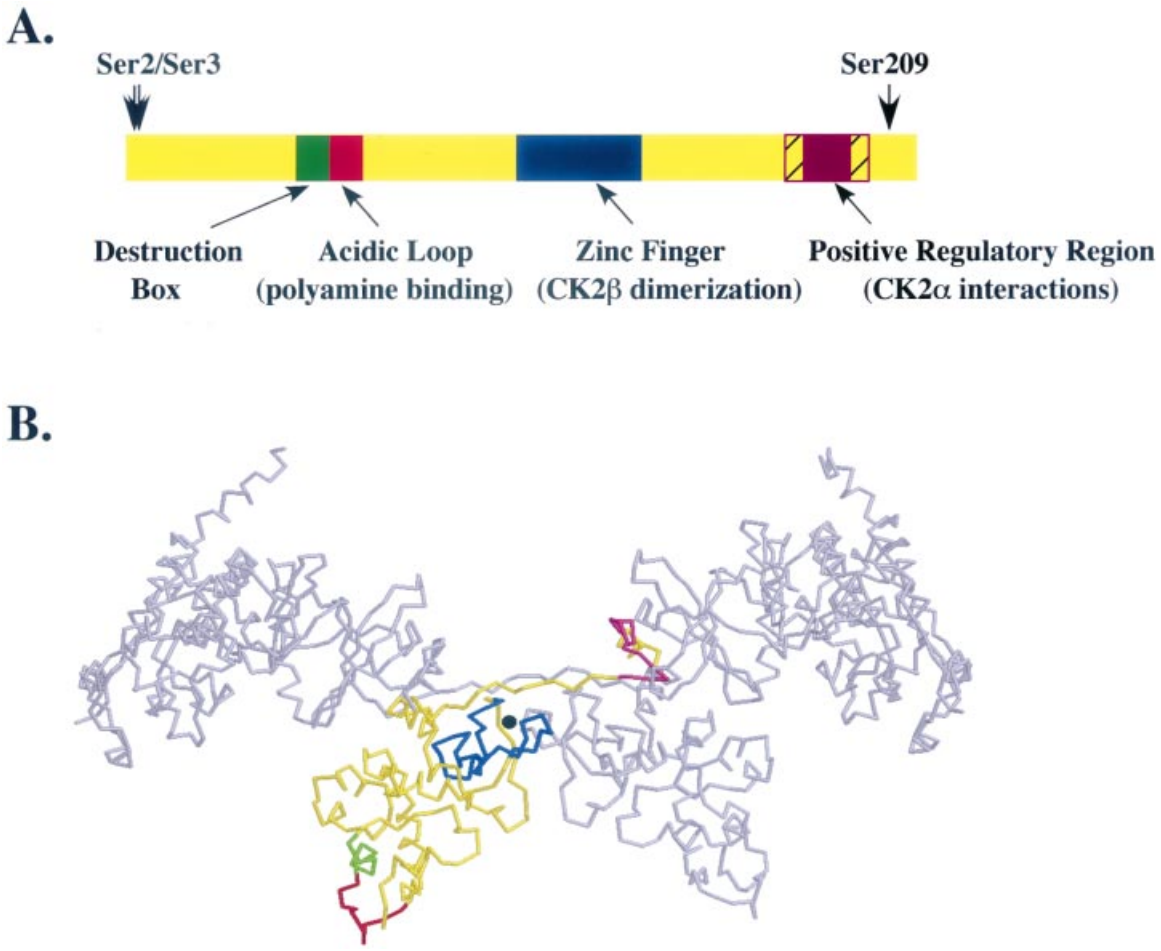

Figure 2 The regulatory CK2β subunit

(**A**) Linear representation of CK2β, illustrating notable elements within its amino acid sequence. These elements include a sequence (Arg^{47}–Asp^{55}) resembling a destruction box (shown in green), an acidic loop (Asp^{55}–Asp^{64}) that is involved in polyamine binding and the possible regulation of CK2 activity (shown in red), a zinc finger (Cys^{109}–Cys^{140}) that mediates CK2β dimerization (shown in blue) and a positive regulatory domain that is involved in interactions with the catalytic subunits of CK2 (shown in magenta). Based on studies with synthetic peptides [77,78], this positive regulatory domain has been defined as a sequence encompassing Asn^{181}–Ala^{203} (indicated by hatched bars). Contacts between CK2α and a sequence (Arg^{186}–Gln^{198}) within this positive regulatory region of CK2β (solid magenta bar) have been identified in the high-resolution structure of CK2 [29]. Additional discussion of these sequences is provided in the text. (**B**) Backbone representation of tetrameric CK2, prepared using RASMOL [206] with structural co-ordinates ([205]; PDB identification number 1JWH) obtained from X-ray crystallography [29]. The orientation of CK2 subunits is the same as illustrated in Figure 1. One CK2β subunit is illustrated in yellow, with each of the notable sequence elements highlighted using the same colours that are used in (**A**). The Zn^{2+} that is involved in the formation of the zinc finger is illustrated as a black dot.

govern the stability of CK2β [73], it remains to be seen whether the destruction box acts in concert with other signals, such as phosphorylation, to regulate the stability of tetrameric CK2 or CK2β itself. Other sequences within CK2β that are noteworthy include a stretch of acidic amino acids encompassing residues 55–64 [12,75,76], a zinc finger containing four cysteine residues (i.e. Cys^{109}, Cys^{114}, Cys^{137} and Cys^{140}) that mediates dimerization of CK2β subunits [26,28,29], and a C-terminal region that positively regulates the catalytic activity of CK2 [77,78].

Based on its similarity to the clusters of acidic amino acids that are typically observed in CK2 substrates, it is tempting to speculate that the sequence comprising residues 55–64 of CK2β (i.e. DLEPDEELED) is reminiscent of the autoinhibitory sequences that have been identified in a number of other protein kinases [79,80]. Several lines of experimental evidence are consistent with the conclusion that this region of CK2β does indeed regulate CK2 activity. For example, polyamines, which have long been known to activate CK2 *in vitro*, bind to this region of CK2β [76,81,82]. Interestingly, mutations that neutralize negative charges within this segment of CK2β abolish stimulation by polyamines and raise the basal activity of the enzyme [76]. Based on these and other observations, a model for the intramolecular regulation of CK2 was proposed whereby this acidic stretch encompassing residues 55–64 of CK2β was postulated to interact with a basic cluster of amino acids on a catalytic subunit of CK2 (i.e. residues 74–80 of CK2α) within the same tetrameric CK2 complex. Although there are attractive features to this model, it is not consistent with the recent crystal structure of tetrameric CK2 [29], which demonstrates that this acidic stretch comprising residues 55–64 of CK2β is located a considerable distance away from the active site of either catalytic subunit (Figure 2). In fact, this acidic stretch is located more than 30 Å from the basic stretch of amino acids comprising residues 74–80 of CK2α [29]. Nevertheless, as may be the case for autophosphorylation, it is conceivable that interactions between residues

55–64 of CK2β and residues 74–80 of CK2α occur through higher-order interactions between CK2 tetramers.

The high-resolution structure of CK2β that was determined by X-ray crystallography demonstrated that a zinc finger anchored by Cys^{109}, Cys^{114}, Cys^{137} and Cys^{140} is responsible for the dimerization of regulatory CK2β subunits [28]. As expected, mutation of Cys^{109} and Cys^{114} to disrupt the zinc finger results in loss of interactions between regulatory CK2β subunits [26]. However, when examined *in vitro* and when expressed in mammalian cells, these mutants also failed to interact with catalytic CK2 subunits. Previous studies had demonstrated that CK2β is synthesized in excess of catalytic CK2 subunits, and that formation of CK2β dimers can occur in the absence of catalytic CK2 subunits [24–28,68,83]. Consequently, the failure of dimerization-incompetent mutants of CK2β to form complexes with catalytic subunits of CK2 may indicate that the formation of CK2β dimers is a prerequisite for the formation of complexes with the catalytic subunits of CK2.

The final segment of CK2β to be highlighted in this discussion is its C-terminal domain, which was initially classified as a positive regulatory domain because of its ability to enhance and stabilize the catalytic activity of CK2 in a manner analogous to that observed with full-length CK2β [77,78]. Studies with synthetic peptides have delineated a sequence encompassing residues 181–203 of CK2β that is responsible for these activities (Figure 2). The direct involvement of this region of CK2β in interactions with CK2α has been confirmed by the high-resolution structure of tetrameric CK2 [29].

CK2-INDEPENDENT FUNCTIONS OF CK2β

An extensive body of work indicates that the regulatory (non-catalytic) CK2β subunit plays an important role in the assembly of tetrameric CK2 complexes, in enhancing the catalytic activity and stability of CK2, and in modulation of the substrate selectivity of CK2 [25,26,68,84,85]. Furthermore, in many cases, it is apparent that CK2β is responsible for docking and/or recruitment of CK2 substrates or potential regulators. In this respect, potential CK2 targets, such as Nopp140, p53, Fas-associated factor-1 (FAF-1), topoisomerase II and CD5, as well as potential CK2 regulators such as fibroblast growth factor-2 (FGF-2), interact with CK2 via interactions with CK2β [86–92].

However, there is a growing body of evidence to suggest that CK2β also performs functions that are distinct from its role as the regulatory subunit of CK2. For example, immunofluorescent localization studies indicate the presence of CK2β in locations where catalytic subunits are not detected [48]. Immunoprecipitation studies from cell and tissue extracts further substantiate the existence of populations of CK2β that are devoid of CK2 catalytic subunits [93]. Utilizing the yeast two-hybrid system, CK2β was identified as an interaction partner of the c-Mos and A-Raf protein kinases, apparently involving the same C-terminal region of CK2β that was shown previously to interact with CK2α [94–97]. Studies performed in *X. laevis* oocytes suggest that, via its inhibitory interactions with c-Mos, CK2β can negatively regulate progesterone-induced maturation [97]. These latter results are consistent with the growth inhibition that is observed upon overexpression of CK2β in yeast [98]. Similarly, ectopic expression of CK2β in mouse 3T3-L1 adipocytes and in CHO cells led to attenuated proliferation resulting from G_2 delay and/or G_2/M arrest [99]. Although all of these results imply that CK2β generally exerts a negative effect on proliferation, it is important to note that CK2β did not dramatically effect proliferation when overexpressed in human osteosarcoma cells or in mouse fibroblasts [100,101]. The reason why the effect of CK2β differs between different cell lines is not known. However, these discrepancies do underscore the complex nature of CK2 and its cellular regulation and functions. Moreover, despite the discrepancies, these results demonstrate that, although a major role for CK2β clearly involves CK2, its CK2-independent functions cannot be overlooked.

REGULATION OF CK2 IN CELLS

A major area of confusion, controversy and excitement within the CK2 field over the years has been the debate over its regulation in cells [102]. The fact that CK2 activity is generally detected in cell or tissue extracts even in the absence of any stimulation or addition of cofactors, or when it is expressed in bacteria, lends itself to the conclusion that CK2 is constitutively active or unregulated. By comparison, beginning in the latter part of the 1980s, studies reporting on the activation of CK2 in response to a diverse array of stimuli were published (reviewed in [102]). As yet, these studies have not yielded any consistent, or general, insights into the mechanisms responsible for the regulation of CK2 in cells. Although it is not yet possible to reconcile precisely all of the opposing views that have appeared, it does appear that a number of distinct mechanisms contribute to the physiological regulation of CK2. Some of the mechanisms that contribute to the regulation of CK2 in cells include regulated expression and assembly, regulation by covalent modification, and regulatory interactions with protein and/or non-protein molecules (summarized in Figure 3). There is also the intriguing observation from the high-resolution structure of tetrameric CK2 (illustrated in Figure 1) that the ATP analogue adenosine 5′-[β,γ-imido]triphosphate occupies the ATP binding site of only one of the catalytic CK2 subunits within the tetramer [29]. Although this situation may result from crystallization of the tetrameric CK2, this result implies that only one catalytic subunit is active at one time. Since the physiological significance of this observation remains unknown, this aspect will not be further discussed.

Regulated expression and assembly of CK2

In the case of the cyclin-dependent kinases, it is evident that kinase activity is absolutely dependent on the presence of regulatory cyclin subunits [103,104]. In this respect, there appear to be a number of intriguing analogies between CK2β and cyclins, including the fact that CK2β modulates the catalytic activity and substrate specificity of CK2 as well as the assembly of CK2 complexes. The existence of a putative destruction box within the sequence of CK2β and the demonstration that CK2β is ubiquitinated and degraded through a proteasomal pathway further emphasizes its potential similarities with cyclins [73]. In accordance with the prospect that CK2 is analogous to cyclin-dependent kinases, it has been reported that CK2 activity oscillates during the cell cycle, although this phenomenon is not universally observed [105,106]. In general, it does appear that CK2 levels correspond to proliferation rate, as cells with higher proliferation rates generally exhibit higher levels of CK2 [107]. In a related vein, CK2α' has been identified as a delayed early gene that is induced following stimulation of quiescent fibroblasts [108]. However, unlike cyclin-dependent kinases, alterations in the activity or expression of CK2 at different stages of the cell cycle do not appear to be absolute, and all CK2 subunits are expressed throughout the cell cycle [106].

As noted above, CK2 has traditionally been considered to be a tetrameric enzyme, with CK2β exerting control over the catalytic activity of CK2 at a number of possible levels. However,

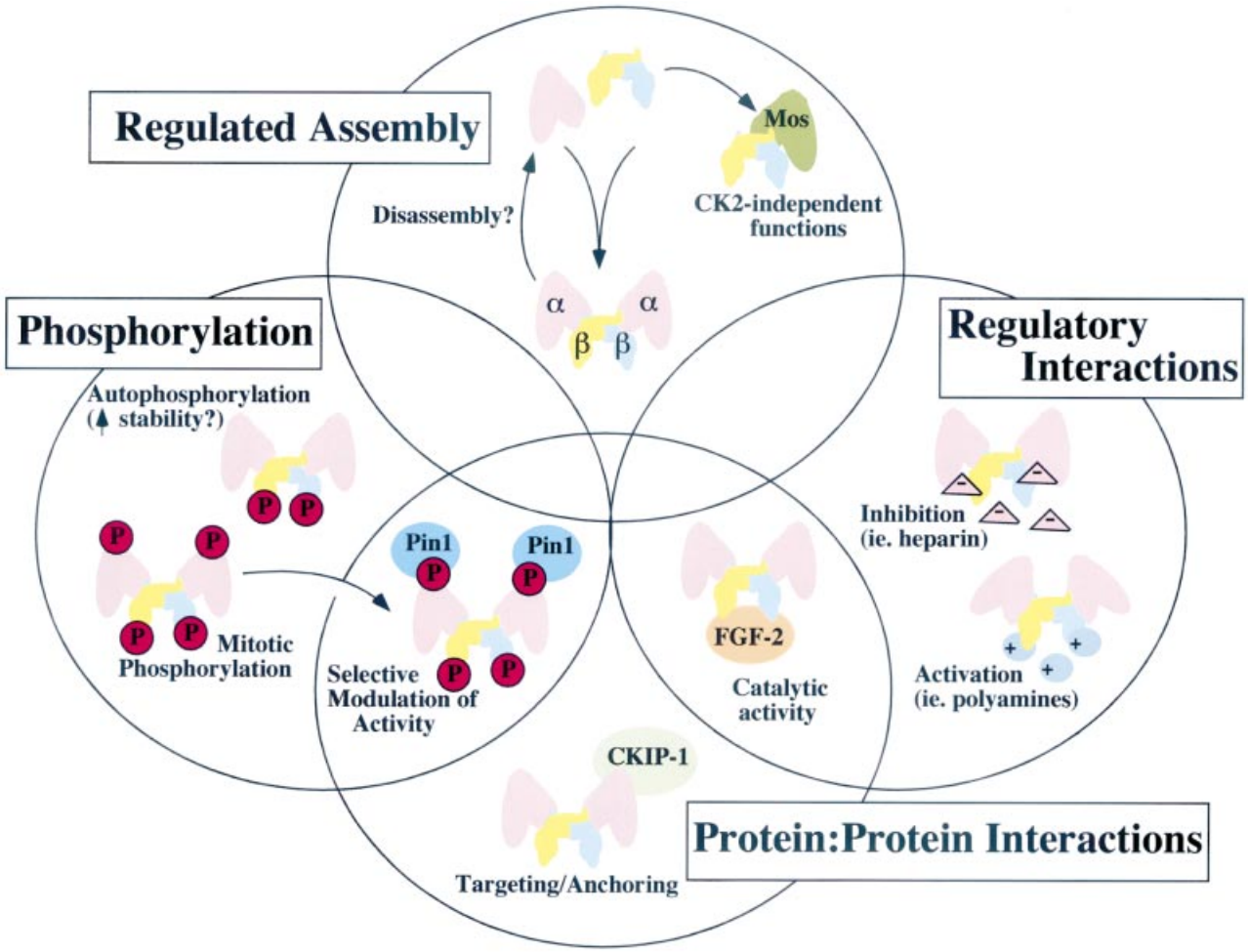

Figure 3 Possible mechanisms of regulation of CK2

As discussed in the text, a number of distinct mechanisms appear to be involved in the regulation of CK2. Selected examples illustrating each of these potential modes of regulation are illustrated. As indicated by the intersection of circles, in some cases CK2 may be subject to more than one possible level of regulation. For example, interactions between CK2 and Pin1 require prior phosphorylation of CK2. These mechanisms are described in more detail in the text. CK2 is illustrated as tetramers or individual subunits, as in Figure 1. Phosphorylation sites on CK2 are indicated by **P** within red circles. Examples of specific proteins that interact with CK2 include Pin1, CKIP-1 and FGF-2, as well as Mos which interacts with CK2β. Polyanionic compounds that may inhibit CK2 (exemplified by heparin) are illustrated by — within pink triangles, and polycationic compounds that may activate CK2 (exemplified by polyamines) are illustrated by + within blue circles.

as is the case with CK2β, there is mounting evidence to suggest that the catalytic subunits of CK2 exist outside tetrameric CK2 complexes. In this respect, it is intriguing that there are substrates, the prototype being calmodulin, that can be phosphorylated by CK2α or by CK2α', but not by tetrameric CK2 [85]. Conventional wisdom, based on studies demonstrating that tetrameric CK2 complexes cannot be dissociated *in vitro* without denaturing agents [109], suggests that the existence of catalytic subunits of CK2 that are devoid of regulatory subunits would simply represent the failure of these subunits to form tetrameric complexes. However, these studies do not exclude the possibility that tetrameric CK2 complexes undergo regulated disassembly in cells. In fact, recent evidence from dynamic localization studies of the individual CK2 subunits provides indications of independent movements of CK2α and CK2β within cells [110]. In a similar vein, the surface contacts between the catalytic and regulatory subunits of CK2 that were revealed by the recent crystal structure of tetrameric CK2 were considerably fewer than the surface contacts typically observed in stable protein complexes [29]. With the prospect that complexed and uncomplexed catalytic CK2 subunits exhibit distinct spectra of cellular targets, it will be important to determine whether CK2 does indeed undergo regulated disassembly and reassembly in cells [85,111].

Phosphorylation of CK2

For many protein kinases, including members of the MAP kinase families of protein kinases, it is apparent that stimulus-dependent phosphorylation of sites within an activation loop is required for their activation [112,113]. In the absence of activating enyzmes, the MAP kinases are not active when expressed as recombinant proteins in bacteria. By comparison, the catalytic subunits of CK2 exhibit robust activity when expressed in bacteria in either the presence or the absence of CK2β [114–116]. In a similar respect, with very few exceptions, there has been limited support for the suggestion that phosphorylation regulates the activity of CK2 in response to cellular stimulation [64,102,117–121]. Collectively, these data indicate that phosphorylation is not absolutely required to activate CK2 in a manner analogous to that seen with MAP kinases. These data do

not, however, exclude the possibility that phosphorylation does participate to some degree in aspects of CK2 regulation.

Examination of CK2 isolated from mammalian cells has led to the identification of a number of physiological phosphorylation sites on both CK2α and CK2β [44,45,64]. In mammalian cells, the β subunit of CK2 is phosphorylated at its autophosphorylation site and at Ser^{209}, a site that is phosphorylated in a cell cycle-dependent manner (Figure 2), while CK2α is phosphorylated in a cell cycle-dependent manner at four sites within its unique C-terminal domain [53,54]. These sites do not appear directly to effect a dramatic change in the catalytic activity of CK2 [38,121]. However, by controlling the stability of CK2β, autophosphorylation may indirectly regulate cellular CK2 activity, a possibility that remains to be rigorously tested [73]. The C-terminal phosphorylation of CK2α may also regulate CK2 indirectly through interactions of phosphorylated CK2α with the peptidyl-prolyl isomerase Pin1 [54]. Interactions between Pin1 and CK2 do not appear generally to influence CK2 activity, but do inhibit the CK2-catalysed phosphorylation of topoisomerase IIα *in vitro*.

There are additional indications that phosphorylation may contribute to the regulation of CK2, although as yet no other sites have been identified that are phosphorylated on CK2 in cells. For example, in the absence of CK2β, the catalytic subunits of CK2 can undergo autophosphorylation at a site (Tyr^{182} in CK2α) that is located within its activation loop [122]. CK2 can also be phosphorylated by the c-Abl protein tyrosine kinase [123] and by members of the Src-family protein tyrosine kinases (L. Pinna, personal communication). While the demonstration that CK2 is tyrosine-phosphorylated promises to yield new insights regarding the integration of CK2 with other signalling pathways, additional studies will be required to define the precise role of these tyrosine phosphorylation events in the physiological regulation of CK2 in cells.

Regulatory interactions

CK2 has typically been classified as a messenger-independent kinase because its activity is not dependent on those small molecules, such as cyclic nucleotides, lipids and calcium, that are typically involved in the activation of second messenger-dependent kinases [124]. However, this classification does not exclude the possibility that small molecules participate in some aspects of CK2 regulation. For example, it has long been known from *in vitro* studies that CK2 is inhibited by negatively charged compounds such as heparin, and activated by positively charged compounds, including polyamines (reviewed in [124]). Although the overall physiological relevance of these latter observations remains unknown, the finding that CK2 levels and activity were elevated in mice with enhanced polyamine levels resulting from forced overexpression of ornithine decarboxylase supports the possibility that CK2 levels can indeed be modulated by polyamines *in vivo* [125]. Furthermore, as mentioned above, it is noteworthy that an overall increase in basal catalytic activity accompanies the loss of polyamine sensitivity that is observed *in vitro* following mutation of the polyamine binding site of CK2β [76]. Although many questions about the precise mode of regulation of CK2 remain, it does appear conceivable that small molecules could indeed participate in aspects of its regulation.

Protein–protein interactions

A large body of evidence indicates that protein–protein interactions represent a major mechanism for the regulation of specific protein kinases [126,127]. For example, proteins that interact with cyclin-dependent kinases, such as $p21^{waf1}$ and $p27^{kip1}$, regulate catalytic activity directly [103,104]. Other interacting proteins do not affect catalytic function directly, but regulate the ability of a particular protein kinase to phosphorylate its cellular targets by functioning as anchoring proteins, scaffold proteins or targeting proteins [128,129]. For example, A-kinase anchoring proteins that are localized to specific subcellular sites play an integral role in regulating the phosphorylation of many substrates of cAMP-dependent protein kinase [128,129]. Since CK2 and cAMP-dependent protein kinase both exhibit the ability to phosphorylate a broad spectrum of cellular targets and are both distributed in a variety of subcellular sites, it is intriguing to speculate that anchoring or targeting proteins may contribute to the regulation of CK2 and its diverse array of apparent cellular substrates.

The identification of several proteins that interact with CK2 is consistent with this conjecture that CK2 may be directly, or indirectly, regulated by interacting proteins. In this regard, it is interesting to consider different categories for some of the proteins that have been identified as CK2-interacting proteins. In the case of proteins such as nucleolin or Nopp140, it is likely that the interactions with CK2 simply reflect enzyme–substrate interactions [86,130]. It has also been shown that CK2 interacts with proteins such as FGF-1 [131], FGF-2 [92], Hsp90 (heat-shock protein 90) [132] and Cdc37 [20,133] that may directly alter or stabilize its catalytic activity. Studies have demonstrated that CK2 also interacts with other proteins, such as tubulin [134], FAF-1 [89] and CKIP-1 [22,53], that may be involved in the targeting of CK2 to specific sites or structures within cells. While the mechanisms responsible for its translocation remain to be defined, recent studies have demonstrated a redistribution of CK2 within the nucleus and to the nuclear matrix in response to heat shock [135,136].

An additional mechanism by which CK2-interacting proteins appear to regulate CK2 activity is illustrated by Pin1 [54] and by the chromatin transcriptional elongation factor (FACT) complex [137]. In the case of Pin1, which interacts with CK2 in a phosphorylation-dependent manner, a selective inhibition of the phosphorylation of Thr^{1342} of topoisomerase IIα by CK2 is observed. By comparison, in response to UV, interactions between CK2 and the FACT complex, which is composed of hSpt16 and SSRP1 (structure-specific recognition protein 1) proteins, result in a selective modulation of CK2 activity that facilitates the phosphorylation of Ser^{392} on p53 [137]. Although the precise effects observed in each of these cases differ, it is evident that interactions between CK2 and specific proteins may lead to the selective modulation of CK2 activity towards individual substrates.

Overall, as illustrated in Figure 3, it is evident that many distinct mechanisms may contribute to the regulation of CK2 in cells. Coupled with the other potential modes of regulation that have been highlighted in this discussion, the observation that specific interacting proteins can selectively modulate CK2 activity towards individual substrates suggests that there may be many independent subpopulations of CK2 within cells. In this respect, it is conceivable that many distinct, independently regulated subpopulations of CK2 exist in cells in order to carry out its myriad of cellular functions.

CK2: OVERVIEW OF CELLULAR FUNCTIONS

It is both exciting and confounding that CK2 appears to reside in a variety of cellular compartments and to participate in the phosphorylation and regulation of a broad array of cellular targets [7–15]. In fact, CK2 has been detected within the nucleus

and cytoplasm, and is also associated with specific structures or organelles, including the plasma membrane, Golgi, endoplasmic reticulum and ribosomes, and has even been detected as an ecto-protein kinase activity on the outer surface of the plasma membrane [8,138–143]. Therefore it may not be surprising that CK2 has been implicated in such a broad array of cellular functions. Rather than attempting to compile a list of all possible cellular functions, this discussion will highlight the importance of CK2 in the context of cell survival, and will discuss the development of strategies for investigating the cellular functions of CK2.

Based on evidence from genetically tractable organisms such as yeast and slime mould, it has been demonstrated that CK2 is essential for viability [20,144]. Disruption of the gene encoding CK2β in mice leads to a failure in development, as does RNAi (RNA interference)-mediated knockdown of CK2β in *Caenorhabditis elegans* [11,145]. Furthermore, there is mounting evidence indicating that CK2 is a component of regulatory protein kinase networks that are involved in various aspects of transformation and cancer. In this respect, abnormally high levels of CK2 have been observed in a number of cancers, including those of the mammary gland [146], prostate [147], lung [148], head and neck [149] and kidney [150]. A striking induction of CK2 was also observed in the lymphocytes of cattle that exhibit leukaemia-like disease following infection with the parasite *Theileria parva* [151]. Elevated expression of CK2α' was also detected by SAGE (serial analysis of gene expression) analysis of a number of metastatic tumours, further re-inforcing the link between CK2 levels and cancer [152]. A more direct link between CK2 and transformation occurs in transgenic mice, where targeted expression of CK2α in T cells and in mammary glands leads to lymphomagenesis and mammary tumorigenesis respectively [146,153]. In addition, CK2 exhibits oncogenic co-operativity when mice with T-cell-specific expression of CK2α were cross-bred with mice overexpressing the c-*myc* or *tal-1* oncogenes, or with p53-deficient mice [153–156]. Co-operation was also observed between CK2 and Ha-Ras in the transformation of Balb/c 3T3 and rat embryo fibroblasts [108]. One striking exception to the positive influence of CK2 on transformation that has been observed in a variety of studies is the demonstration that CK2 inhibits transformation induced by oncogenic Ras [52]. Despite this discrepancy, it is evident that CK2 has functions associated with transformation.

Although it is evident that alterations in CK2 levels can have profound effects in the context of transformation and cancer, the molecular basis for these effects still remains incompletely understood. In this respect, candidate cellular CK2 substrates include proto-oncogene products such as c-Myc [157], c-Myb [158] and c-Jun [159], tumour-suppressor gene products such as p53 [30,137] and BRCA1 (breast cancer susceptibility gene 1) [160], transciptional regulators such as Max [161,162], Cut [163], PU.1/IRF4 (interferon regulatory factor 4) [164,165] and Six1 [166], as well as components of the canonical Wnt pathway [146,167–169]. Additional work on these and other candidate CK2 targets ultimately promises to yield a mechanistic understanding of how CK2 participates in transformation.

IDENTIFICATION OF *BONA FIDE* CELLULAR TARGETS OF CK2

Based on the minimal CK2 consensus motif that has been identified, it seems that examination of almost any protein sequence with motif-scanning software yields one or more candidate CK2 phosphorylation sites. Given its ubiquitous expression and its localization to many sites within the cell, it does not often require much imagination to develop a reasonable working hypothesis postulating that CK2 participates in the regulation of a particular protein of interest. However, as has been described elsewhere for many other protein kinases [170], it is important that a number of criteria be fulfilled before a protein is designated as a *bona fide* physiological substrate for CK2. First of all, it is essential that sites that are phosphorylated *in vitro* by CK2 are actually shown to be phosphorylated in cells. Secondly, alterations in the phosphorylation state of the CK2 sites should occur upon changes in the cellular activity of CK2. With the availability of tissue-purified and recombinant CK2 and the utilization of traditional or emerging strategies for the identification of phosphorylation sites, it is often relatively straightforward to fulfil the first criterion. The somewhat unique ability of CK2 to utilize GTP as a phosphate donor in place of ATP [171] can often be helpful in these studies.

However, owing in large part to the broad array of cellular functions of CK2 and to complexities regarding its regulation in cells, it can frequently be much more challenging to satisfy the second criterion. In particular, there are no well established strategies that can be universally exploited to manipulate the cellular activity of CK2. However, there are recent developments in the design of selective CK2 inhibitors and in the use of genetic strategies for manipulating CK2 in cells that offer promise for overcoming these challenges. For example, new selective inhibitors of CK2, such as 4,5,6,7-tetrabromobenzotriazole (TBB), may provide a better degree of specificity than did previous generations of inhibitors [172]. In addition to TBB, a number of other compounds, including apigenin [173], 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) [174] and emodin [156], have been utilized as inhibitors of CK2 in cells. However, as with many inhibitors of many other protein kinases [170], questions regarding their precise specificity and mechanism(s) of action in cells remain. This cautionary note is raised particularly for those compounds that are competitive with respect to ATP, since ATP is the substrate for all members of the protein kinase family in addition to a vast array of other cellular enzymes. In the case of one member of the stress-activated p38 kinase pathway, an elegant approach for validating the specific effects of one its inhibitors was achieved by engineering of an inhibitor-resistant form of the kinase [175]. In this respect, it is noteworthy that mutagenesis studies demonstrated that the sensitivity of CK2 to TBB or to emodin is diminished several-fold by mutation of a bulky hydrophobic residue that is present within the catalytic site of members of the CK2 family of enzymes (i.e. Val^{66} in CK2α), but absent from the vast majority of other protein kinases [172,176]. Whether mutants of CK2 that are resistant to TBB or other compounds will be useful in cells remains to be seen, but it can readily be envisaged that the development of such mutants could be a major boon for the CK2 field.

Recent advances in the manipulation of CK2 using genetic strategies also offer promise for the development of systematic approaches for the identification of its cellular substrates and elucidation of its cellular functions. As in many fields, elegant studies in genetically tractable organisms, such as yeast, have provided the most definitive insights to date into the cellular functions and targets of CK2 [20]. In fact, a decade ago [177], topoisomerase II was classified as the first *bona fide* cellular target of CK2 when it was demonstrated, in yeast with temperature-sensitive CK2, that topoisomerase II was hypophosphorylated at sites identical to sites that were phosphorylated by CK2 (and not other protein kinases) *in vitro*. Current approaches for the systematic elucidation of cellular substrates of CK2 in mammalian cells still lack the precision that can be achieved through gene replacements in organisms such as yeast. Genetics-based strategies that are currently available for the

systematic elucidation of cellular targets or functions of CK2 in mammalian cells include the overexpression of catalytically active forms of CK2 to augment cellular CK2 levels [46,101]. However, since many cells contain relatively high basal levels of CK2, overexpression approaches do not necessarily yield dramatic changes in cellular levels of CK2 and may not effect significant changes in the phosphorylation of its targets. In some cases, it may be preferable to employ interference strategies, which include the use of catalytically inactive mutants of CK2 [46,101] or of antisense or RNA interference strategies to block CK2-mediated signalling events [178–181]. Again, the utility of these strategies may be hindered to some degree by the high basal levels of endogenous CK2. Furthermore, in the case of antisense strategies, it is necessary to pay close attention to the similarities and differences between CK2α and CK2α' and to consider the long apparent half-life of CK2 [68]. Despite the limitations of these strategies, progress towards the establishment of model systems for the systematic investigation of CK2 has been made. For example, mammalian cells with tetracycline-regulated expression of CK2 have been established and offer new opportunities for altering CK2 levels in cells in order systematically to identify changes in the phosphorylation state of cellular proteins that may be directly or indirectly regulated by CK2 [46,100]. Since it is possible to manipulate the expression of CK2 in an isoform-specific manner, these strategies offer new opportunities to evaluate the specific functions of each CK2 isoform in a manner that is not possible using pharmacological inhibitors such as TBB that cannot distinguish between different forms of CK2.

In summary, it can be relatively straightforward to identify candidate target substrates for CK2. However, given the complexities of CK2 and its regulation and functions, validation of these candidates as *bona fide* CK2 targets can still be a daunting task, especially in organisms where gene disruption and replacement strategies are not yet routine.

CK2 AND CELLULAR DECISIONS OF LIFE AND DEATH

Role in cell cycle regulation and cell division

Evidence for essential roles of CK2 at various stages during the cell cycle continues to mount. For example, genetic studies in yeast indicate that CK2 is required for progression through both the G_1/S and G_2/M transitions [20]. In mammalian cells, cell cycle progression can be inhibited by antisense oligonucleotides directed against CK2α or CK2β [181], by microinjection of anti-CK2 antibodies and by inhibitors of CK2 [166,182,183]. As in yeast, these studies suggest that CK2 is required at multiple transitions in the cell cycle (including G_0/G_1, G_1/S and G_2/M). Additional evidence for roles for CK2 in the G_2/M transition and mitosis come from the observation that CK2 is associated with the mitotic spindle and with centrosomes [47,48]. The demonstration that CK2α and CK2β are phosphorylated in mitotic cells provides a further indication that CK2 is a regulatory participant in events associated with this stage in the life of a cell [44,64]. As noted above, the mitotic phosphorylated form of CK2 interacts with Pin1, an essential regulator of cell division and a replication checkpoint [54,55,184]. This latter finding adds another potential level of regulation to the involvement of CK2 in the control of cell division.

Collectively, the data cited above indicate that CK2 does participate in the regulation of various stages of the cell cycle, presumably through the phosphorylation and regulation of proteins that have important functions associated with cell cycle progression. In this regard, many cell cycle regulatory proteins, including $p34^{cdc2}$, cdc34 and topoisomerase II, have been identified as likely physiological targets of CK2 [185–188].

A Specificity of Caspases vs CK2

Group I (Caspases 1,4,5)	W-E-H-D ↓
Group 2 (Caspases 2,3,7)	D-E-X-D ↓
Group 3 (Caspases 6,8,9)	(I/V/L)-E-X-D ↓
CK2	(S/T)-X-X-(D/E/pS/pY)

B Specific CK2-regulated Caspase Targets

Substrate	Sequence	Enzyme
Max	-I-E-V-E ↓ S-D-E-E-	Caspase 5
Bid	-E-D-E-L-Q-T-D ↓ G-S	Caspase 8
HS1	?	Caspase 3
Connexin 45.6	-S-D-E-V-E ↓ G-P	Caspase 3

Figure 4 CK2 regulates the cleavage of caspase targets

(**A**) Consensus sequences for caspases [199] and for CK2 [31] are illustrated. Acidic residues within these recognition sequences are highlighted in pink. Caspase cleavage sites are indicated with arrows. Residues phosphorylated by CK2 are boxed in green. (**B**) Specific examples of proteins where phosphorylation by CK2 has been shown to inhibit caspase-mediated cleavage. Acidic residues, CK2 phosphorylation sites and caspase cleavage sites are highlighted as in (**A**). A CK1 phosphorylation site that may act co-operatively with the CK2 phosphorylation site in Bid is highlighted in yellow. Note that Bid is cleaved at aspartic acid [191], whereas Max [190] and connexin 45.6 [192] are both cleaved at glutamic acid. The cleavage site in HS1 [193] has not yet been determined. The specific caspase affected for each protein is indicated.

However, with few exceptions that include the mitotic phosphorylation of topoisomerase II in yeast and in mammalian cells [177,187,188] and the mitotic phosphorylation of the transcription factor Six1 in mammalian cells [166], precise information on when individual targets are phosphorylated by CK2 during the cell cycle remains very limited. In the case of topoisomerase IIα in mammalian cells, mitotic phosphorylation was detected using mitosis-specific MPM-2 (mitotic protein monoclonal-2) and 3F3/2 phospho-epitope antibodies [187,188]. The intriguing observation that CK2 can regenerate a number of the MPM-2-reactive phospho-epitopes that are lost following phosphatase treatment of mitotic extracts clearly suggests that additional proteins are mitotic substrates for CK2 [188].

Role of CK2 in cell survival and apoptosis

As noted above, gene disruption experiments in yeast and slime mould demonstrate that CK2 is essential for viability [20,144]. A failure of kinase-inactive CK2 to restore viability in yeast indicates that it is the catalytic activity of CK2, and not just the presence of CK2 proteins, that is required for viability [20,189]. In mammalian cells, forced expression of kinase-inactive CK2α or CK2α' also compromises cell proliferation [46,101]. Interestingly, in human osteosarcoma U2-OS cells with tetracyline-regulated expression of CK2 [46], cell proliferation and viability were compromised by induced expression of catalytically inactive CK2α'. By comparison, induced expression of catalytically inactive CK2α in these cells was without effect on proliferation or viability, suggesting that CK2α' may have unique functions associated with the control of proliferation or viability. In a similar vein, the predisposition to apoptosis that is observed in the germ cells of male CK2$\alpha'^{-/-}$ mice reinforces the notion

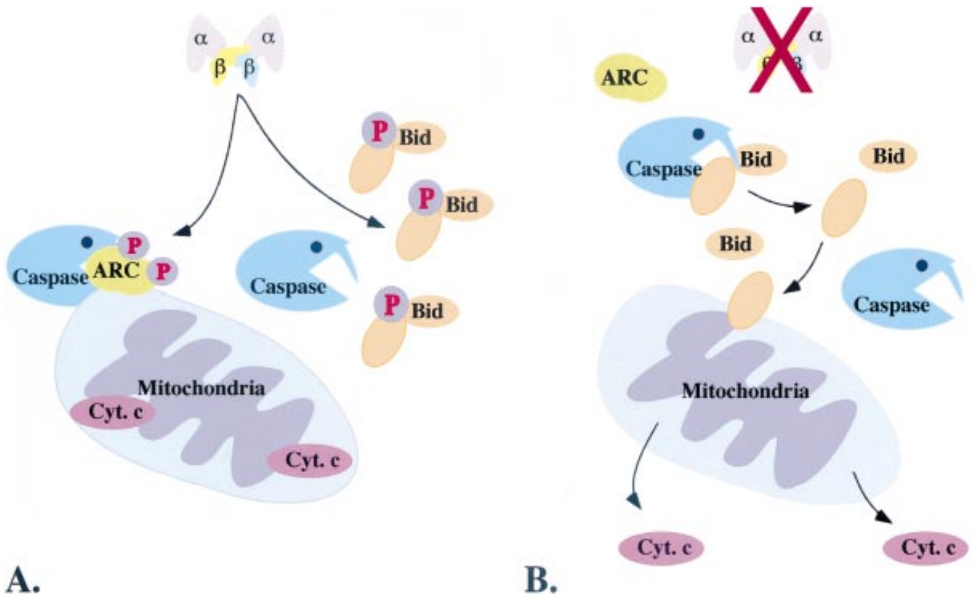

Figure 5 Dual role of CK2 in the regulation of apoptosis

(**A**) Under survival conditions, CK2 phosphorylates proteins such as ARC and Bid. When phosphorylated by CK2, ARC is targeted to mitochondria, where it inhibits caspase 8. Bid is resistant to cleavage by caspase 8 when it is phosphorylated by CK2. (**B**) Under apoptotic conditions and/or when CK2 is compromised, CK2 phosphorylation sites on proteins such as Bid and ARC are not phosphorylated. Under these conditions, ARC is not targeted to mitochondria and does not inhibit caspase 8. In the absence of phosphorylation, Bid is susceptible to cleavage by caspase 8. The subsequent translocation of Bid to the mitochondria is followed by release of cytochrome *c* (Cyt. c) that results in the amplification of caspase activation. As discussed in the text, additional proteins, including Max and HS1, become susceptible to caspase-mediated cleavage when they are not phosphorylated by CK2.

that CK2α' has a role associated with cell viability that cannot be compensated for by CK2α [41].

Although a mechanistic understanding of how CK2 supports viability remains far from complete, recent evidence linking CK2 to apoptosis has yielded intriguing insights (reviewed in [11]). For example, in response to apoptotic stimuli, Max, the transcriptional partner of the c-Myc proto-oncogene product, undergoes caspase-mediated degradation subsequent to an apparent dephosphorylation at CK2 phosphorylation sites [190]. Parallel experiments performed *in vitro* demonstrate that phosphorylation of Max by CK2 *in vitro* protects it from caspase-mediated cleavage. An analogous role for CK2 in modulation of susceptibility to caspases has also been observed with Bid, a pro-apoptotic member of the Bcl-2 family [191], with the gap junction protein connexin 45.6 in the lens [192] and with the haematopoietic lineage cell-specific protein 1 (HS1) [193] (Figure 4). In all cases, phosphorylation by CK2 protects these proteins from caspase-mediated degradation. A complementary mechanism for the regulation of caspases by CK2 has recently emerged with the demonstration that phosphorylation by CK2 is required for the apoptotic protein ARC (apoptosis repressor with caspase recruitment domain) to exert its inhibitory activity towards caspase 8 [194]. Together with the ability of CK2 to protect individual proteins from caspase-mediated cleavage, this latter observation suggests that CK2 may have general anti-apoptotic functions (Figure 5). This latter conjecture is supported by the observation that increased expression of CK2 protects cells from drug-induced apoptosis [195]. In a similar vein, inhibitors of CK2 have been reported to trigger apoptosis and to increase the susceptibility of cancer cells to chemotherapeutic agents or apoptotic stimuli [193,196,197]. Collectively, these results suggest that CK2 is indeed a regulatory participant in pathways that control cell survival and apoptosis. A further implication of these results is that the increased expression of CK2 that is frequently observed in cancer [146–152] may result in enhanced survival of these cells due to the potential anti-apoptotic function of CK2.

In view of the fact that phosphorylation by CK2 can protect specific proteins from caspase-mediated degradation, it is noteworthy that there is a striking similarity between the recognition sequence for degradation by caspases [198,199] and the consensus motif for phosphorylation by CK2. In fact, as illustrated in Figure 4, it is evident that numerous sequence patterns can conform to the minimal sequence requirements for recognition by caspases and by CK2. Thus it is conceivable that CK2 functions to some degree as a sensor of cell integrity that exerts a general cell survival or anti-apoptotic function through its ability to phosphorylate numerous proteins that would be destined for caspase-mediated degradation during apoptosis. According to such a model, events that compromise the expression of CK2 or its activity would lead to decreased phosphorylation of its target proteins, which in turn could lead to a release of caspase inhibition as well as to an increase in the susceptibility of proteins to caspase-mediated degradation (Figure 5). Although there are alternative explanations, the demonstration that forced expression of catalytically inactive CK2α' or treatment of cells with the selective CK2 inhibitor TBB are each sufficient to induce apoptosis is consistent with this model [46,193].

Additional support for a potential role for CK2 in the modulation of events that control decisions relating to cell survival or cell death come from studies establishing a link between CK2 and stress signalling pathways (reviewed in [11]). For example, genetic studies in *Schizosaccharomycyes pombe* and *S. cerevisiae* indicated that CK2 has functions associated with responses to DNA damage [200,201]. In the context of how CK2 might be regulated in response to cellular stresses, one

particularly intriguing aspect of this work is the demonstration that an apparent disassembly of tetrameric CK2 complexes accompanies the induction of DNA damage in *S. cerevisiae* [201]. There is also evidence that CK2 is involved in stress signalling in mammalian cells. For example, the tumour suppressor p53 is phosphorylated in response to UV irradiation at a residue (Ser^{392} in human cells) that is phosphorylated *in vitro* by CK2 [137,202]. CK2 has also been implicated in responses to other stresses, including heat shock, anisomycin, arsenite and tumour necrosis factor-α (TNF-α) [135,136,203]. As with UV irradiation, increased phosphorylation of Ser^{392} on p53 has been reported in response to anisomycin and TNF-α, suggesting that there may be common elements to the participation of CK2 in responses to these different agents. However, as noted above, interactions between CK2 and the FACT complex are involved in responses to UV irradiation [137]. By comparison, it has been reported that an interaction between CK2 and activated p38 MAP kinase is important for the increased phosphorylation of Ser^{392} that occurs in response to anisomycin, arsenite and TNF-α treatment [203]. CK2, together with p38 MAP kinase, also appears to be required for execution of a spindle checkpoint that results in a G_2 arrest in mammalian cells that have been treated with nocodazole [179]. In addition to the loss of spindle checkpoint activity, inhibition of CK2 activity or depletion of CK2 using antisense strategies results in attenuated apoptotic responses in these cells. While the latter result is not entirely compatible with the anti-apoptotic functions of CK2 that have been proposed above, it does provide further evidence that CK2 does indeed participate in cellular decisions of life and death. Furthermore, this apparent duality of function displayed by CK2 is yet one more example of the complexities involving its regulation and functions that are likely to continue to characterize the CK2 field.

CONCLUSION AND FUTURE PROSPECTS

Evidence from biochemical experiments and from recent high-resolution crystal structures of CK2 [28,29] have dramatically improved our appreciation of the architectural arrangement of subunits within tetrameric CK2 complexes, and have provided many new insights into the mechanisms by which CK2 is regulated in cells. From these studies, it is evident that the regulation of CK2 in cells is complex, with a number of distinct mechanisms contributing to this regulation (see Figure 3). Given the participation of CK2 in a myriad of cellular events and the complex nature of its regulation, it is also apparent that a number of discrete, independently regulated populations are likely to exist within cells. Despite these advances, a precise understanding of CK2 and its biological functions remains far from complete. In fact, although an extensive list of potential physiological substrates has emerged, rigorous proof that most of these proteins are *bona fide* cellular substrates for CK2 is lacking. With the exception of genetically tractable organisms such as yeast [20], the CK2 field has traditionally been hindered by the absence of reliable strategies for its precise manipulation in living systems. However, new generations of CK2 inhibitors [172] and the development of mammalian cell lines [46,100] and animal models with altered expression of individual forms of CK2 [11,41,146,153] offer new prospects for systematically addressing the functions of CK2 in these systems. Furthermore, new genomic and proteomic strategies for the investigation of changes in gene expression profiles [204] and for the identification of alterations in protein expression or phosphorylation profiles [5] promise to uncover the underlying basis for CK2-dependent changes in biological function that are observed in these models. Systematic strategies for the characterization of networks of interacting proteins [56–58] and for the dynamic visualization of proteins in living cells [110] promise to enhance further our understanding of each of the different forms of CK2. It can be readily envisaged that these studies will dramatically expand the list of physiological targets of CK2, and will link CK2 to a variety of additional cellular processes. As this new information emerges, it may not become any more straightforward to define concisely the precise regulation and functions of CK2. However, this information may help to provide rational resolutions for some of the confusion and controversy that has confounded the field.

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