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Casein kinase II in signal transduction and cell cycle regulation

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Abstract

Casein kinase II is a protein serine/threonine kinase that is ubiquitously distributed in eukaryotes. Molecular cloning studies and protein sequence analysis of purified proteins have demonstrated the existence of two related, but distinct, isozymic forms of its catalytic subunit in mammals and birds. At present, the precise role of the individual casein kinase II isoforms in biological responses is poorly understood. However, a great deal of evidence indicates that casein kinase II is an important component of signalling pathways that control the growth and division of cells. In particular, casein kinase II is known to phosphorylate, and in several cases, regulate the activity of a variety of regulatory nuclear proteins including nuclear oncoproteins, transcription factors, and enzymes involved in other aspects of DNA metabolism. In this review, we will summarize evidence relating to the involvement of casein kinase II in signal transduction events that are relevant to cell proliferation. (Mol Cell Biochem **127/128:** 187–199, 1993)

Key words: casein kinase II, protein kinase, protein phosphorylation, signal transduction, transcriptional regulation, cell cycle

Introduction

The phosphorylation and dephosphorylation of proteins is a major mechanism controlling a variety of cellular processes which include events relating to the growth and proliferation of cells [1–3]. Furthermore, genetic and biochemical studies have demonstrated the existence of networks of protein kinases that are involved in the regulation of cell division and in the transmission of regulatory signals [1–7]. One component of these networks is casein kinase II (CKII), a protein serine/threonine kinase that is ubiqitously distributed in eukaryotic cells [8–11]. While its precise role in biological responses remains poorly characterized, a great deal of recent evidence indicates that this enzyme is involved in the regulation of proliferative events. Since the biochemistry of CKII and a detailed discussion of its substrates have been the topic of previous reviews [8–11], this review will focus on the most recent information that indicates that CKII is an important component of regulatory signalling pathways relevant to the growth and division of cells.

Subunit composition of casein kinase II

In most organisms, CKII is a tetrameric enzyme composed of two α (and/or α′) subunits and two β subunits, although exceptions to the tetrameric structure may exist in some organisms (reviewed in [8, 9]). Through

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                                                                  * *     *
Human1      MSGPVPSRARVYTDVNTHRPREYWDYESHVVEWGNQDDYQLVRKLGRGKYSEVFEAINIT      -60
Chicken1    ..................................................._._...._.....
Xenopus     ....................D..........................._._...._.....
Human2      MPGPAAG......AE..SL.S.......A..PS..............._._...._.....
Chicken2    MPGPAAG......AE..SL.S.......A..PS..............._._...._.....

                 *               *
Human1      NNEKVVVKILKPVKKKKIKREIKILENLRGGPNIITLADIVKDPVSRTPALVFEHVNNTD      -120
Chicken1    ........_.............._.....................................
Xenopus     ........_.............._.....................................
Human2      ...R..._.............._V.........T...K.I.T......K.......YI....
Chicken2    ...R..._.............._V.........T...N.I.T......K.......YI....

                                                  * *  *              * *
Human1      FKQLYQTLTDYDIRFYMYEILKALDYCHSMGIMHRDVKPHNVMIDHEHRKLRLIDWGLAE      -180
Chicken1    ..........................................._._.._.............._._...
Xenopus     ..........................................._._.._.............._._...
Human2      ......I...F........L.........K....._._.._.....QQK....._._...
Chicken2    ......I...F........L..............._._.._.....QQK....._._...

                              **              *    *
Human1      FYHPGQEYNVRVASRYFKGPELLVDYQMYDYSLDMWSLGCMLASMIFRKEPFFHGHDNYD      -240
Chicken1    ....................__.............._.....__.....................
Xenopus     ....................__.............._.....__.....................
Human2      ....A..............__.............._.....__.........R......Q....
Chicken2    ....A..............__.............._.....__.................Q....

Human1      QLVRIAKVLGTEDLYDYIDKYNIELDPRFNDILGRHSRKRWERFVHSENQHLVSPEALDF      -300
Chicken1    ..................................................................
Xenopus     ..................................................................
Human2      ............E..G.LK..H.D...H......Q.......N.I....R.........L
Chicken2    ............DE..G.LK..H.....H......Q.......N.I....R......V..L

                    *
Human1      LDKLLRYDHQSRLTAREAMEHPYFYTVVKDQARMGSSSMPGGSTPVSSANMMSGISSVPT      -360
Chicken1    ..........._.............PI.........N...........S..........
Xenopus     ..........T_........D.....PI....S..AALIC.VAAH.SVAPV*
Human2      ..........Q_....K.........P...E.SQPCADNAVLSSGLTAAR*
Chicken2    ..........Q_....K.........P...E.SQPSSENAVLSSGLTTAR*

Human1      PSPLGPLAGSPVIAAANPLGMPVPAAAGAQQ*                                -391
Chicken1    ............S.TTT............*
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Fig. 1. Alignment of deduced amino acid sequences of CKII α and CKII α′ subunits from vertebrates. The following sequences are shown: human α (human1) [14,15], human α′ (human2) [15], chicken α (chicken1) [16], chicken α′ (chicken2) [16] and Xenopus laevis α [27]. Numbering of amino acids corresponds to the numbering of human α. Residues that are conserved in all members of the protein serine/threonine kinase family [19] are underlined and indicated with an asterisk.

molecular cloning studies [12–16] and direct protein sequence analysis of purified proteins [17, 18], the existence of an isozymic form of the α subunit (designated α′) has also been demonstrated in yeast, mammals and birds. These studies clearly indicate that the isozymic forms of CKII are products of distinct genes. Sequence analysis demonstrates that the conserved consensus motifs of protein kinase family members [19] are all found within the deduced sequences of the α and α′ subunits (see Fig. 1). In mammals and birds, the α subunits (391 amino acids) and α′ subunits (350 amino acids) exhibit a very high degree of identity. For example, over the 330 N-terminal residues of their deduced sequences (containing all conserved protein kinase motifs) the human α and α′ subunits exhibit approximately 85% identity (identity exceeds 90% when conservative substitutions are considered). Interestingly, the C-terminal domains of α and α′ are completely unrelated suggesting that functional differences between the two isoforms may result from differences within these domains (see also below). Sequence comparisons also demonstrate a very high degree of conservation for the α and α′ subunits of CKII between species (see Fig. 1). Chicken [16] and human [14, 15] α subunits exhibit 98% identity while the α′ subunits from these species exhibit 97% identity. By comparison with the α subunits of CKII, the β subunit does not share any identity with the catalytic or regulatory subunits of other protein kinases [20–27]. The CKII β subunit has considerable sequence identity with the protein encoded by the stellate gene of Drosophila melonagaster, but the functional significance of this observation is unknown [28]. While its precise functions remain poorly understood, the CKII β subunit displays extraordinary conservation; remarkably the deduced amino acid sequences of human (as reported by Jakobi *et al.*, and Teitz *et al.* [21, 25]), mouse [24, 26] and chicken [16] β are identical (see Fig. 2). Furthermore, the β subunit of Xenopus laevis differs from the β subunit of birds and mammals by only a single amino acid. Clearly, the exceptional degree of conservation exhibited by the α, α′ and β subunits of CKII suggests that functional properties of CKII have been conserved throughout evolution.

As implied from sequence homology with other protein kinases, the isolated α subunits of CKII have protein kinase activity [29–31]. A precise role for β has not yet been established, but it appears to have a role in enhancing or stabilizing the kinase activity of the α subunit [32–36]. The β subunit is known to be autophosphorylated *in vitro* [8, 9] and in cells [37]. However, the function of autophosphorylation has not been determined since deletion of the autophosphorylation site has no apparent affect on the ability of recombinant β to associate with, and enhance the kinase activity of α [36]. Biosynthetic labeling studies [38] indicate that nearly all of the newly synthesized α subunit is rapidly incorporated into holoenzyme and that significant quantities of free α subunit do not exist in exponentially growing cells. By comparison, β appears to be synthesized in excess over α and is more slowly incorporated into holoenzyme. The α and β subunits that assemble to form holoenzyme appear to have a very long half-live, in contrast to free β which is rapidly degraded. Collectively, the experiments that have been summarized above suggest that β is an important component of CKII; however, its precise functions remain elusive.

Substrate specificity of casein kinase II

The substrate specificity of CKII has been determined by identifying the sites that are phosphorylated in its substrate proteins and by the systematic analysis of synthetic peptides (reviewed in [8, 9]). Collectively, these studies indicate that the consensus sequence for CKII is Serine(or Threonine)-X-X-Acidic where the acidic amino acid can be glutamic acid, aspartic acid, phosphoserine [39–41] or phosphotyrosine [42], but apparently not phosphothreonine [41, 42]. Although the composition of the X amino acids has not been comprehensively examined it is apparent that certain amino acids, i.e. proline in the 1st X position) could prevent substrate phosphorylation by CKII [43]. In general, it seems that the best CKII substrates appear to have several acidic residues on the C-terminal side of the phosphorylatable residue. It should also be noted that CKII phosphorylation sites that do not conform to the Serine-X-X-Acidic motif have been identified [8, 9, 44]. The observation that phosphorylated amino acids can serve as specificity determinants for CKII is of interest because it suggests that CKII will phosphorylate some proteins only after prior phosphorylation by a distinct protein kinase, an example of hierarchal phosphorylation (as reviewed by Roach [45]).

Localization of casein kinase II

CKII is known to phosphorylate a variety of regulatory cytosolic and nuclear proteins, a result that suggests that

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Human     MSSSEEVSWISWFCGLRGNEFFCEVDEDYIQDKFNLTGLNEQVPHYRQAL    -50
Mouse     ..................................................
Chicken   ..................................................
Xenopus   ..................................................

Human     DMILDLEPDEELEDNPNQSDLIEQAAEMLYGLIHARYILTNRGIAQMLEK   -100
Mouse     ..................................................
Chicken   ..................................................
Xenopus   ..................................................

Human     YQQGDFGYCPRVYCENQPMLPIGLSDIPGEAMVKLYCPKCMDVYTPKSSR   -150
Mouse     ..................................................
Chicken   ..................................................
Xenopus   ..................................................

Human     HHHTDGAYFGTGFPHMLFMVHPEYRPKRPANQFVPRLYGFKIHPMAYQLQ   -200
Mouse     ..................................................
Chicken   ..................................................
Xenopus   ..................................................

Human     LQAASNFKSPVKTIR*                                     -215
Mouse     ..............*
Chicken   ..............*
Xenopus   ............M.*
```

Fig. 2. Alignment of amino acid sequences of CKII β subunit from vertebrates. The following sequences are shown: human β [21, 25], mouse β [24, 26], chicken β [16] and Xenopus laevis β [27]. The sequence of human β reported by Heller-Harrison *et al.* [22], has alanine at residue 194.

CKII may be located in different cellular compartments. Indeed, biochemical studies relying on measurements of CKII activity had indicated that it is localized within the cytoplasm, the nucleus and may be associated with other structures within cells [8, 9]. In recent years, more precise analyses have been performed by immunofluorescent and immunoelectron microscopy. These studies have confirmed that CKII is localized within the nucleus and the cytoplasm. However, there are very significant differences in the observations made by different investigators. Initial observations made independently in different laboratories using polyclonal antibodies raised against the CKII holoenzyme demonstrated that CKII is localized within the nucleolus, an observation that is consistent with the observation that nucleolar proteins can be phosphorylated by CKII [46, 47]. Alterations in the nuclear/cytoplasmic distribution of CKII have also been documented. More than one laboratory has suggested that the nuclear population of CKII is more prominent in proliferating cells than it is in quiescent cells, and that there may be a regulated nuclear translocation of the enzyme [47, 48]. The latter suggestion is supported by the observation that microinjection of β-specific monoclonal antibodies results in an apparent decrease in the nuclear accumulation of CKII [49].

The localization of the individual subunits of CKII has also been investigated using antipeptide antibodies specific to the different subunits of CKII. The studies of Yu *et al.* [50], indicate that CKII α may be primarily cytosolic and CKII α′ primarily nuclear in G_1 cells. Interestingly, when cells entered S phase, CKII α′ was reported to leave the nucleus. In contrast, the studies of Krek *et al.* [51], suggested that the α, α′ and β subunits are all localized predominantly to the nucleus. Both studies indicat-

ed that a population of CKII became associated with the mitotic spindle in dividing cells. It is difficult to explain the discrepancies between the two studies. However, it may be significant that Yu *et al.* [50] did not utilize α′ specific antibodies for their studies and instead determined the localization of CKII α′ by examining the difference in immunoreactivity observed when using antibodies that recognized both α and α′ and antibodies that exclusively recognized α. Krek *et al.* [51] also noted that β-specific antibodies detected a signal (perhaps centrosomes) that was not detected with α or α′-specific antibodies. Despite indications that β may have functions independent of α or α′, it should be noted that the subunits of CKII have normally been found in tetrameric complex.

Clearly, there appear to be a number of unresolved issues regarding the precise localization of CKII species within cells. At least some of the apparent discrepancies that have been reported may result from the fact that different laboratories have used different cell lines, antibodies and fixation procedures for immunolocalization experiments. Nevertheless, it does appear that CKII is, at least in part, a nuclear enzyme that could play a significant role in the transmission of regulatory signals within the nucleus.

Casein kinase II in signal transduction

There is a great deal of circumstantial evidence that suggests that CKII is an important regulator of proliferative events. In particular, studies indicate that the activity of CKII is elevated in rapidly proliferating cells as compared to quiescent cells and in proliferating tumour tissue compared to non-tumour tissue [52, 53]. At least in some instances, it appears likely that increases in CKII activity reflect corresponding increases in levels of CKII protein. More direct evidence that CKII has an important role in proliferative events comes from studies in the yeast Saccharomyces cerevisiae. Disruption of the CKA1 gene (encoding α) or the CKA 2 gene (encoding α′) does not produce an obvious phenotype [12, 13]. By comparison, disruption of both CKA1 and CKA2 results in a complete loss of viability which can be restored by introduction of a cDNA encoding a functional α subunit from Drosophila, but not a kinase-deficient Drosophila α subunit [54]. These results indicate that the kinase activity of CKII is essential for viability and demonstrate functional conservation between the α subunits of different organisms. Similarly, it appears that the CKII α subunit is essential for the vegetative growth of Dictyostelium discoideum, since attempts to obtain disruptants of the gene encoding the protein were not successful [55].

Studies in mammalian cells also support the suggestion that CKII is an important component of signalling pathways relevant to the control of proliferation. Pretreatment of primary human IMR-90 fibroblasts with antisense oligonucleotides for the α and β subunits of CKII delayed entry of these cells into S phase following mitogenic stimulation [56, 57]. In a similar vein, microinjection of β-subunit specific monoclonal antibodies partially inhibited serum-induced proliferation perhaps by preventing the nuclear translocation of CKII [49]. The role of CKII in mitogenic signalling has also been investigated by microinjection of purified protein into mammalian cells. Microinjection of CKII into quiescent rat embryo fibroblasts by Gauthier-Rouviere *et al.* [58] induced c-fos expression presumably through a mechanism involving phosphorylation of serum response factor (SRF). By comparison, microinjection of CKII was shown by Lin *et al.* [59] to suppress induction of AP-1. Although, as noted by Lin *et al.*, that there may be examples of AP-1 induction without c-fos expression, it would appear that the results of the microinjection studies are contradicatory. Collectively, these results suggest that CKII may play a role in regulating proliferative events. However, before the precise roles of CKII in cell regulation are completely understood, a number of apparent discrepancies must be resolved.

Phosphorylation of regulatory nuclear proteins

In recent years, a role for CKII in the regulation of nuclear events through the phosphorylation of regulatory nuclear proteins has rapidly emerged. Particularly prominent on its list of probable physiological substrates are a number of nuclear oncoproteins, a tumour suppressor protein, transcription factors and proteins involved in DNA metabolism. For a partial list of its potentially important nuclear substrates see Table 1. For all of these proteins, evidence suggests that CKII can phosphorylate sites that are known to be phosphorylated in cells. In some cases, phosphorylation appears to affect functional properties of the proteins, whereas in other cases (i.e. c-Myc [60, 61], N-Myc [62, 63], c-Erb A [64]) effects of phosphorylation have not yet been observed. Since the phosphorylation of many of these proteins has

been thoroughly reviewed, the forthcoming discussion will emphasize the most recent findings that suggest a role for CKII in the control of functional properties of nuclear regulatory proteins.

As indicated in Table 1, various forms of the Myc family of oncoproteins (including c-Myc and N-Myc) have been shown to be phosphorylated by CKII. In the case of c-Myc, CKII phosphorylation sites have been localized to two regions of the protein; a central region of the protein and a C-terminal region that is adjacent to its basic helix-loop-helix/leucine zipper domain [60]. Mutation of the CKII phosphorylation sites within the latter region to non-phosphorylatable (alanine or aspartic acid) residues did not have a significant effect on Myc activity when analysed in a co-transformation assay [61]. However, since CKII sites within this region are highly conserved throughout evolution, it remains possible that CKII phosphorylation of c-Myc could have some functional effects that have not yet been characterized. Recent evidence has demonstrated that c-Myc functions as a transcriptional regulator when part of a heterodimeric complex with another basic helix-loop-helix/leucine zipper containing protein designated Max [65, 66]. Two forms of Max, generated by alternate splicing, have been identified; both forms can be phosphorylated *in vitro* by CKII at sites (Serine2 and Serine11) that are phosphorylated in cells [66, 68]. Initial studies of Berberich and Cole [67] indicated that CKII phosphorylation of Max could inhibit the DNA binding activity of Max:Max homodimers, but not Myc:Max heterodimers. However, a more detailed kinetic analysis indicates that CKII phosphorylation of Max increases the exchange rate for DNA binding of Max:Max homodimers and Myc:Max heterodimers [68].

Phosphorylation by CKII has been shown to negatively affect the DNA binding activities of two nuclear oncoproteins, c-Myb [69] and c-Jun [59]. In the case of c-Myb, CKII phosphorylation occurs at serine residues (Serine11 and Serine12) that are deleted in oncogenically-activated forms of Myb [69]. This observation suggests that the loss of regulatory phosphorylation sites may contribute to oncogenic potential. However, there is as yet no direct evidence that indicates that CKII phosphorylation of c-Myb can regulate its DNA binding activity in cells. Furthermore, mutation of the CKII phosphorylation sites in c-Myb has no apparent effect on its transactivation of a reporter gene (M. Oelgeschläger and B. Lüscher, unpublished observations). Phosphorylation of c-Jun, a component of the AP-1 transcription factor complex, by CKII occurs at two residues (Threonine231 and Serine 249) that appear to negatively regulate DNA binding activity [59]. Microinjection of synthetic peptide inhibitors of CKII into living cells resulted in induction of AP-1 activity. The effect of inhibitory peptides could be overcome by increasing cellular CKII levels by microinjection of purified enzyme. Microinjected CKII also inhibited phorbol ester-induced increase in AP-1 activity. Collectively, these results suggest that CKII has a role in suppressing AP-1 activity in resting cells by inhibiting the DNA binding activity of c-Jun.

In addition to its apparent role in the negative regulation of transcription factor activity, CKII has also been shown to activate the DNA binding activity of other

Table 1. Regulatory nuclear proteins phosphorylated by casein kinase II

Protein	Function	Effects of phosphorylation	Refs.
c-Myc	transcription factor	?	60, 61
N-Myc	transcription factor	?	62, 63
Max	transcription factor	inhibits DNA binding of max/max homodimers	67
		increases on/off rate for DNA binding of max/max and myc/max	68
c-Myb	transcription factor	alters DNA binding	69
c-Jun	transcription factor	inhibits DNA binding	59
SRF	transcription factor	increases on/off rate for DNA binding	58, 70–73
c-ErbA	transcription factor	?	64
PU.1	transcription factor	promotes interaction with NF-EM5	80
p53	tumour suppressor	activates DNA binding	75
		mutation of CKII site abolishes anti-proliferative potential	74
UBF	nucleolar transcription factor	apparent transactivation of RNA polymerase I dependent transcription	81, 82
E7	transforming protein of HPV type 16	mutation of CKII sites decreases transformation potential	89, 90
large T	transforming protein of SV40	increases nuclear transport	87
topoII	DNA topoisomerase	increases decatenation activity	84, 85
DNA ligase 1	ATP-dependent ligation of DNA	increases activity	83

transcription factors. The DNA binding activity of serum response factor (SRF), a transcription factor that binds to the serum response element within the promoter region of the c-fos proto-oncogene, was initially shown to be dependent on phosphorylation by CKII, a result that suggested CKII had the potential to regulate c-fos expression by controlling the phosphorylation of SRF [70]. Moreover, increasing the levels of CKII activity in quiescent fibroblasts by microinjection of purified enzyme resulted in enhanced phosphorylation and stimulation of c-fos expression [58]. However, other studies suggest that the role of CKII in regulating SRF activity and c-fos expression may not be so straightforward. Detailed kinetic analyses [71, 72], indicate that CKII actually enhances the on/off rate for DNA binding by SRF without significantly altering binding affinity. Also, recent studies by Manak and Prewes [73] demonstrate that there is no change in the extent of SRF phosphorylation by CKII when cells are stimulated with serum. The latter observations suggest that phosphorylation of SRF by CKII may not be the primary regulatory event that controls c-fos expression. Nevertheless, the CKII phosphorylation sites on SRF are known to be phosphorylated in cells and are known to be highly conserved throughout evolution. Thus, it is plausible that CKII may control certain functions of SRF, or protein complexes containing SRF, that have not yet been examined.

Another protein that appears to require CKII phosphorylation in order to bind DNA is p53, a tumour suppressor protein with a postulated role as a transcriptional regulator [74, 75]. CKII has been shown to associate with p53 and can phosphorylate a residue near the C-terminus of p53 that does not conform to the Serine-X-X-Acidic consensus for CKII phosphorylation [76–78]. Recent studies demonstrate that phosphorylation of this residue by CKII can activate the binding of p53 to DNA [75]. Furthermore, mutation of the CKII site in p53 abolishes its antiproliferative activity suggesting that CKII may play a role in regulating the activity of p53 *in vivo* [74]. Given that the activity of p53 could be regulated during the cell cycle [79], these observations imply that CKII, or perhaps its interactions with p53, could also be regulated during the cell cycle. While the precise functions of p53 remain poorly defined, it may be involved in arresting cell cycle progression in response to DNA damage. Interestingly, the cDNA encoding the β subunit of CKII was shown to confer a degree of UV resistance to Xeroderma Pigmentosum cells of the complementation group D [25]. Although the mechanism by which this effect was induced remains uncharacterized, the results suggest that CKII, or perhaps the free β subunit, may have a role in some aspect of DNA repair. At this stage, there is no evidence that indicates any kind of connection between the role of CKII in DNA repair and its interactions with p53. However, it is tempting to speculate that there may be some functional relationship between these events.

Phosphorylation by CKII also appears to regulate the transactivation activity of a B cell specific complex containing the PU.1 and NF-EM5 proteins that interact with a region of the enhancer of the κ immunoglobulin chain [80]. Sequence specific DNA-binding by the NF-EM5 protein requires complex formation with PU.1; phosphorylation of PU.1 promotes association with NF-EM5. Furthermore, mutation of the CKII phosphorylation site on PU.1 dramatically diminished the ability of the NF-EM5/PU.1 complex to stimulate transcription of a reporter gene in S194 plasmacytoma cells.

Recent studies also suggest that CKII phosphorylation may be involved in stimulating the transactivating activity of UBF, a nucleolar protein that functions as a transcription factor for the RNA Polymerase I dependent transcription of ribosomal RNA genes [81, 82]. The extent of UBF phosphorylation and its transactivating activity (but not its DNA binding activity) are at higher levels in proliferating cells as compared to resting cells. Since CKII appears to be the major kinase responsible for UBF phosphorylation, these results imply that CKII activity is higher in proliferating cells. CKII also appears to be involved in the activation of DNA ligase I [83] and DNA topoisomerase II [84, 85]. Interestingly, phosphorylation of the latter protein was significantly diminished at the non-permissive temperature in yeast containing temperature-sensitive CKII suggesting conclusively that CKII is indeed a physiological kinase that targets topoisomerase II [85]. In addition, phosphorylation of topoisomerase II by CKII appears to be maximal at the G_2/M transition suggesting that the activity of CKII could be regulated during the cell cycle.

Endogenous cellular proteins do not appear to be the only substrates that are affected by CKII. Large T antigen [86, 87], one of the transforming proteins of SV40 and E7 [88–90] one of the transforming proteins of human papilloma virus type 16 can be phosphorylated by CKII at sites that are known to be phosphorylated in cells. Evidence from Rihs *et al.* [87], suggests that CKII phosphorylation of large T at residues near its nuclear localization sequence may be involved in regulating its transport to the nucleus. In the case of E7, mutation of its CKII phosphorylation sites diminishes its ability to

transform NIH 3T3 cells or BRK cells [89, 90]. The mechanism by which E7 is regulated by CKII remains unknown since the binding of E7 to the Rb gene product was unaffected by mutation of CKII phosphorylation sites [89, 90].

In summary, a great deal of evidence indicates that CKII has a very significant role in the regulation of nuclear protein functions. In particular, the involvement of CKII in the phosphorylation of proteins involved in the control of gene expression demonstrates that this enzyme is a key component of regulatory pathways controlling proliferative events.

Regulation of CKII in cells

There are indications from studies in mammalian fibroblasts [91] as well as amphibian and echinoderm oocytes that the activity of CKII may be regulated at different stages in the cell cycle [92–95]. There is also evidence that CKII may be activated by various stimuli (see Table 2) including serum, insulin, IGF-1, EGF, bombesin, tumour necrosis factor and phorbol ester [91, 96–104, 108, 109]. However, the activation of CKII is not a universal response of cells to these stimuli, since measured changes in CKII activity are not always observed even using the same stimulus [97, 105–107] (see Table 2). Furthermore, in most instances the observed increase in CKII activity is relatively modest, and in some cases cannot be observed until a cell extract has been subjected to chromatographic fractionation. There have also been suggestions that the activity of CKII is constitutively expressed in cells and is not subject to acute regulation [7–9]. To gain a full understanding of CKII and its role in biological events, discrepancies regarding its activation in cells need to be resolved.

At present, the precise mechanisms by which CKII is regulated in cells remains poorly characterized, but it does appear that several factors could be involved. There have been indications that CKII is activated by phosphorylation. Most notably, Ackerman *et al.* [102], demonstrated that treatment of human A431 cell extracts with alkaline phosphatase could result in a return of EGF-stimulated CKII activity to basal levels. The protein kinases responsible for phosphorylation of CKII in response to EGF were not identified. It has also been shown that purified CKII can be phosphorylated and activated *in vitro* by purified $p34^{cdc2}$ [110] or by purified protein kinase C [111]. In contrast to these results, it has been shown that the activity of purified CKII can be increased by dephosphorylation [112]. Since phosphorylation sites were not analysed in any of the studies detailed

Table 2. Regulation of casein kinase II in cells

Stimulus	Cell type or tissue	Effect	Refs.
Insulin	mouse 3T3-L1 cells	increase	96, 99
	rat H4-IIE cells	increase	96
	mouse Balb c/3T3 cells	increase*	97
	rat adipocytes	increase*	100
	rat adipocytes	no effect	105
	rat liver	decrease	107
IGF-1	mouse Balb c/3T3 cells	increase*	97
Epidermal growth factor	human A431 cells	increase	96, 101, 102
	Balb c/3T3 cells	no effect	97
	human A431 cells	no effect	37
	mouse Swiss 3T3 cells	no effect	106
serum	human WI.38 cells	increase	91
bombesin	mouse Swiss 3T3 cells	increase*	103
Tumor necrosis factor	mouse Swiss 3T3 cells	increase*	104
	mouse L929 cells	increase*	104
phorbol ester	rat BRK cells	increase	108
	human A431 cells	no effect	101
phorbol ester and ionomycin	mouse B cells	increase	109

* Increase in CKII activity was measurable only after chromatographic fractionation of extracts.

above, it is not possible to resolve apparent contradictions. To address the issue systematically, we examined the phosphorylation state of CKII in A431 cells and demonstrated that the β subunit of CKII is phosphorylated at an autophosphorylation site (Serine2, Serine3) and at a residue (Serine209) that is phosphorylated *in vitro* by $p34^{cdc2}$ [37]. We subsequently demonstrated that the α (but not α′) and β subunits of CKII are dramatically phosphorylated in chicken BK3A cells and human Jurkat cells arrested at mitosis [113], a result that suggests very clearly that CKII could be a physiological substrate for $p34^{cdc2}$ (and/or other protein kinases that are activated at mitosis). Comparative phosphopeptide maps indicate that the mitotic sites on CKII-α can be phosphorylated *in vitro* by purified $p34^{cdc2}$. Notably, the mitotic phosphorylation sites on the CKII α subunit are localized to its unique C-terminus, suggesting that this domain is involved in cell cycle dependent regulation of the CKII-α isozyme. Although it appeared that nearly all of the CKII α subunit was phosphorylated in mitotic chicken BK3A cells, we did not measure any apparent change in CKII activity in extracts, or immunoprecipitates, prepared from interphase or mitotic populations of cells [113]. This result suggests that the mitotic phosphorylation of CKII does not directly alter its catalytic activity. Nevertheless, since the mitotic phosphorylation of CKII occurs to high stoichiometry, it is likely that certain functions or properties of the CKII-α isozyme are regulated by its cell cycle-dependent phosphorylation.

As discussed previously, independent studies have shown that the nuclear population of CKII becomes more prominent following the stimulation of resting cells to re-enter the cell cycle suggesting that there is a regulated nuclear translocation of CKII. Also, immunofluorescence localization studies indicated that a population of CKII became associated with the mitotic spindle in dividing cells. Although the factors that control the localization of CKII within cells remain uncharacterized, these studies suggest that changes in the phosphorylation of substrate proteins could be mediated by altering the distribution of CKII within cells without directly altering its catalytic activity. This mode of regulation would be analogous to the targeting of other protein kinases (i.e. cAMP-dependent protein kinase [114], $p34^{cdc2}$ [115]), and phosphatases [3] that are regulated by association with regulatory subunits that control accessibility to different substrates.

In vitro, the activity of CKII is dramatically affected by compounds including polyanionic inhibitors such as heparin and polycationic activators such as spermine and polylysine (reviewed in [8, 9]). However, the role of these compounds in regulating CKII in cells is not yet known. It has been demonstrated that CKII can aggregate *in vitro* (under conditions of physiological ionic strength) to form ordered linear filaments [116]. As yet, the filaments have not been observed in living cells, and the effects of filament formation on kinase activity have not been precisely determined. A number of other cellular proteins (including tubulin [117], p53 [76–78], hsp90 [118–119], and an unidentified 49kDa protein [120]) have also been shown to interact with CKII. Associated proteins could directly regulate the activity of CKII, specify its localization within cells, or could simply be substrate proteins. The interaction with tubulin may be related to the interaction of CKII with the mitotic spindle [50, 51]. Furthermore, it will be of interest to determine whether the mitotic phosphorylation of CKII [113] is involved in controlling its interactions with the mitotic spindle. The association of p53 with CKII has been independently observed by different laboratories [76–78] and appears to be related to the involvement of CKII phosphorylation in activation of DNA binding by p53 [74, 75]. Association of CKII with hsp90 *in vitro* appears to result in kinase activation and inhibition of CKII filament formation [119]. A more thorough understanding of how (or if) the functions of CKII are controlled by its interactions with other proteins requires further analysis.

In summary, a number of potential factors or mechanisms by which CKII may be regulated in cells have been identified. However, as yet there is no consensus on how its functional properties are precisely controlled.

Perspectives

Based on its ubiquitous distribution and exceptional evolutionary conservation, it would appear that CKII plays a vital role in fundamental cellular processes. However, despite the rapid accumulation of evidence that supports a role for CKII in the regulation of proliferative events, its precise functions are poorly understood and a number of issues remain unresolved. In particular, although it has been demonstrated that CKII can phosphorylate a number of regulatory nuclear proteins, there is (with the possible exception of topoisomerase II [85]) very little evidence that indicates when and where CKII phosphorylates its substrates in cells during different stages in the cell cycle or following stimulation with various agents. Without this information it is difficult to determine how CKII actually regulates the functions of

particular proteins in cells. This information is also required to resolve apparent discrepancies regarding its subcellular localization and its regulation (or lack of regulation) in response to various stimuli or at different stages of the cell cycle. Further study is also required to determine whether the isozymic forms are functionally redundant or whether they have specialized functions. By resolving many of these issues, we may ultimately define the position(s) occupied by CKII in the regulatory protein kinase networks that control the growth and division of cells.

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