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From birth to death: The role of protein kinase CK2 in the regulation of cell proliferation and survival

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Abstract. Protein kinase CK2 is a serine/threonine kinase with a multitude of protein substrates. The enzyme is ubiquitously expressed in mammalian cells, where it functions in a variety of cellular processes, including cell cycle progression, apoptosis, transcription, and viral infection. While the importance of CK2 in the mammalian life cycle is undisputed, the regulatory mechanisms coordinating its numerous functions remain elusive. In this review, we focus on the various roles of CK2 in the mammalian cell, with particular attention on its functions through the stages of the cell cycle and during the decision to undergo cell death. We highlight how these roles are controlled in part through direct transcriptional regulation by CK2, and how the constitutive activity of CK2 can be hijacked in the case of viral infection. Finally, we discuss possible ways in which these functions are integrated to allow the cell to respond appropriately in the presence of multiple signals. (Part of a Multi-author Review)

Keywords. CK2, phosphorylation, cell cycle, apoptosis, transcription, virus.

Introduction

Protein phosphorylation has long been recognized as an important post-translational modification regulating cellular processes [1]. Emphasizing the importance of this modification is the existence of 518 distinct protein kinases in the genome [2], and the estimate that one third of cellular proteins are phosphorylated [3], often at several distinct sites [4]. Proper regulation of phosphorylation events is crucial to the proper function of cellular signalling pathways, and loss of regulation in these pathways underlies many human diseases, including cancer [5]. Consequently, the enzymes that regulate protein phosphorylation in cells, namely protein kinases and phosphatases, have emerged as promising therapeutic targets [6]. Protein kinase CK2 [7], a small family of protein serine/threonine kinases that is overexpressed in multiple forms of cancer and has oncogenic properties in mice and cultured fibroblasts [8], represents one of the protein kinase families that has attracted attention as potential targets for therapy. In this review, we will outline a variety of the diverse and often interconnected roles of CK2 that underlie its participation in disease processes, including cell cycle progression, induction of apoptosis, transcription, and its involvement as a target in viral infection. We also discuss the perspective whereby CK2 may be viewed as a regulatory linker, acting to consolidate often conflicting signals from various stimuli into the appropriate cellular response.

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The CK2 Family

Originally discovered in 1954 [9], CK2 is a family of enzymes that in humans consists of two catalytic subunits, termed CK2α and CK2α′, and one regulatory subunit, CK2β. Despite being the products of

different genes [10, 11], the catalytic subunits share a high amount of sequence similarity [12], differing only in their C-termini [13]. CK2β has no extensive homology to any other known proteins [14], making its role difficult to decipher. Typically, CK2 is found in mammalian cells as a tetramer, consisting of two regulatory subunits and two catalytic subunits [15, 16]. CK2 has been distinguished by its ability to phosphorylate serine and threonine residues proximal to acidic amino acids with a minimal consensus sequence of S/T-X-X-Acidic [17], as well as its unique ability to use either ATP or GTP as a phosphate donor [18].

CK2 is ubiquitously expressed in a variety of cell types and tissues, and is considered to be constitutively active, as it is not subject to the strict on/off regulation of other kinases, such as MAP kinases and Cyclin-dependent kinases (Cdks). However, there exist several, more subtle mechanisms by which CK2 activity can be regulated or focused on one or more substrates while excluding other substrates, including localization, phosphorylation, and protein-protein interactions [19, 20].

CK2 in the cell cycle

CK2 has been implicated in every stage of cell cycle progression, and catalyzes the phosphorylation of several proteins crucial to the successful production of daughter cells. In yeast, genetic studies have shown requirements for CK2 for progression through the G1/S and G2/M transitions [21]. In mammalian cells, antisense oligonucleotides against CK2 subunits, microinjection of CK2 antibodies, and inhibitors of CK2 can all inhibit cell cycle progression, suggesting that mammalian cells require CK2 for G0/G1, G1/S, and G2/M transitions [22–24]. Studies outlining the interactions and effects of CK2 activity on cell cycle proteins have further confirmed the importance of CK2 throughout the cell cycle. Cell cycle progression is largely mediated by the controlled activation and deactivation of Cdks, including Cdk4/Cyclin D at the G1/S transition, and Cdk1/Cyclin B at the G2/M transition [25]. These kinases are activated at the appropriate times in part by the activity of the Cdk-activating kinase (CAK), an enzyme consisting of the Cdk7, Cyclin H, and MAT1 (ménage à trois 1) [26]. This master regulator of cell cycle progression is itself regulated by CK2. In this respect, the CK2α subunit forms a complex with and phosphorylates Cyclin H at serine 315 [27, 28]. This phosphorylation event has no effect on CAK complex formation, but is critical for full CAK activity [28].

G1/S

The signal to begin DNA replication and prepare for cell division is transduced from extracellular growth signals through the G1 Cdks, leading to hyperphosphorylation of pRB and transcription of vital cell cycle genes due to release of E2F transcription factor inhibition [29]. Once the initial stimulus for growth has been transduced to the G1 Cdks, CK2 has additional roles in regulating the initiation of DNA replication and preparation for division. In general, the role CK2 plays in the G1/S phase of the cell cycle serves not to encourage cell growth but to monitor it, both as an integral part of the DNA damage checkpoint, where it regulates p53 and p53 regulatory proteins [30–36], and by interacting with and phosphorylating regulatory proteins involved in G1/S checkpoint signalling, including Cdk-inhibitory proteins [37–39].

Perhaps one of the most well-studied roles for CK2 in G1/S is its regulation of the tumour suppressor protein p53, a transcription factor involved in DNA damage signalling that can elicit both cell cycle arrest and induction of apoptosis [29]. p53 is phosphorylated by CK2 at serine 392 in response to UV radiation-induced DNA damage, resulting in increased DNA binding and transcriptional activation [30, 40]. In response to DNA damage caused by UV light, CK2 forms an interaction with the FACT complex (consisting of SSRP1 and hSPT16), resulting in increased CK2 activity towards p53 [31, 40]. SSRP1 is also a substrate of CK2, and phosphorylation decreases SSRP1 binding to DNA, halting transcription in the event of DNA damage [32]. In undamaged cells, p53 is constantly produced and degraded, and the degradation of p53 is induced by MDM-2 (murine double minute clone 2), which targets p53 for processing by the 26S proteasome. In the presence of cellular stress, the interaction between MDM-2 and p53 is disrupted, leading to stabilization of p53, which can then elicit a cellular response [29]. MDM-2 is also a substrate of CK2, which phosphorylates it at serines 260 [35], 267 [36], and 269 [33, 35].Phosphorylation at serine 269 has been shown to decrease MDM-2 binding to pRB, and phosphorylation-site mutants cause altered growth when expressed in cells [34]. CK2 phosphorylation at serine 267 leads to reduced ability to direct p53 degradation [36]. Treatment of cells with the CK2 inhibitor TBB (4,5,6,7-tetrabromo-2-azabenzimidazole) leads to induction of p53 and its transcriptional target genes [35], providing additional evidence of the importance of CK2 regulation of p53 signalling.

Another mechanism by which CK2 regulates G1/S signalling is through interaction and phosphorylation of Cdk inhibitory proteins. $p21^{WAF1/CIP1}$, a potent

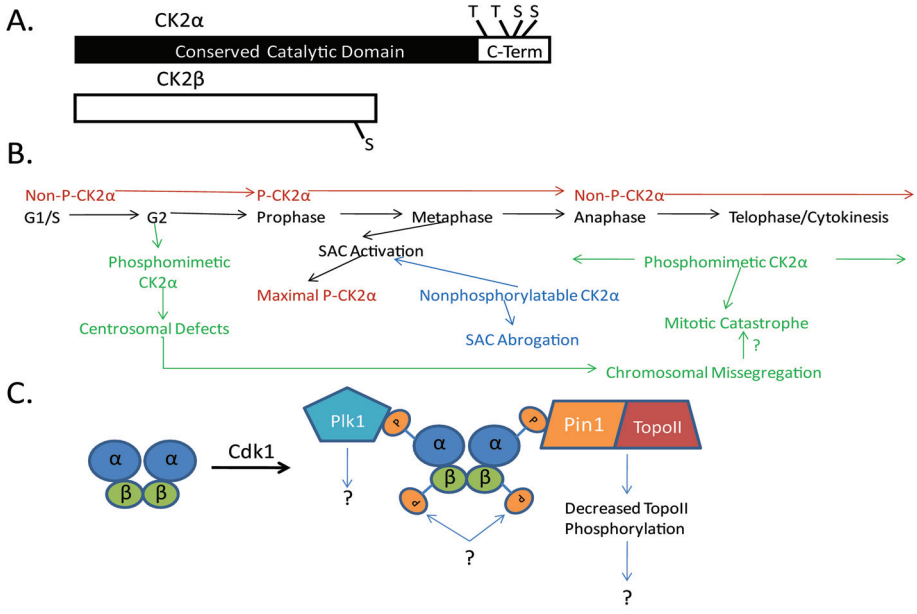

Figure 1. CK2 phosphorylation in mitosis. (*A*) Schematic representation of CK2α and CK2β with mitotic phosphorylation sites (threonines 344 and 360, serines 362 and 370 in CK2α, serine 209 in CK2β) highlighted. (*B*) Model for temporal regulation of CK2α phosphorylation based on experiments using phosphorylation site mutants. (*C*) Model for CK2 mitotic phosphorylation sites as docking sites for mitosis-specific protein-protein interactions. After phosphorylation of CK2 by Cdk1, the CK2α C-terminal phosphorylation sites may be bound by the prolyl isomerase Pin1. Complex formation between Pin1, Topoisomerase II, and phosphorylated CK2α results in decreased mitotic phosphorylation of Topoisomerase II by CK2 [67]. The mitotic kinase Plk1 can also bind to the C-terminal CK2α phosphorylation sites [61]; however, no function has been elucidated for this interaction. Phosphorylation-dependent interacting proteins have not as yet been identified for CK2β.

inhibitor of Cdks whose expression is induced by p53 activation [29], binds to CK2β [37]. CK2 can phosphorylate $p21^{WAF1/CIP1}$ in a CK2β-dependent manner, indicating that CK2β acts to target the catalytic subunits to this substrate [38]. $p27^{KIP1}$, another Cdk inhibitor, is also phosphorylated by CK2 in a CK2β-dependent manner [39]. The effects of CK2 phosphorylation of these substrates has not been determined, but suggests that CK2 can regulate progression through G1/S though multiple mechanisms.

G2/M

The onset of mitosis is associated with a wave of regulatory phosphorylation, and CK2 itself is one of the affected proteins. CK2β is phosphorylated in a cell cycle-dependent manner by the main mitotic cyclin-dependent kinase, Cdk1, at serine 209 [41]. The function of this phosphorylation event remains unknown. The CK2α catalytic subunit is also phosphorylated in a cell cycle-dependent manner by Cdk1, at four residues in its unique C-terminus (threonines 344 and 360 and serines 362 and 370) [42, 43] (Fig. 1A). The presence of these sites indicates independent regulation of the CK2 catalytic subunits in the cell cycle, and a specialized role for CK2α (or α2β2 tetrameric forms) in mitosis. Since these phosphorylation sites do not directly affect CK2 activity, it is hypothesized that they may contribute to subtle regulation of CK2, by forming binding sites for interacting proteins and/or targeting CK2 towards favourable substrates or away from unfavourable substrates in the context of mitotic progression. Through the development of phosphospecific antibodies against the four CK2α phosphorylation sites, we have shown that phosphorylation of CK2α occurs mainly during prophase and metaphase. Furthermore, disruption of these phosphorylation sites leads to centrosomal amplification, abrogation of the spindle assembly checkpoint (SAC), and induction of mitotic catastrophe [150]. On the basis of these observations, we theorize that proper temporal regulation of CK2α phosphorylation is required to avoid these defects (Fig. 1B). The requirement for proper mitotic regulation of CK2 through phosphorylation suggests a crucial role in mitotic progression.

Several lines of evidence point to specific roles for CK2 in the G2/M transition and mitosis. CK2 colocalizes with the mitotic spindle and centrosomes [44, 45], and many proteins involved in mitosis are interacting partners and/or substrates of CK2, including β-Tubulin[46], Cdc25B [47],Tau [48], Condensin [49], PP2A [50], and Microtubule-associated proteins

1A and 1B [48]. Through sequential phosphorylation of Wee1 with Plk1 and Cdk1, CK2 phosphorylation leads to the degradation of Wee1 and onset of mitosis [51]. Attenuation of CK2 activity or knockdown of CK2 subunits has been shown to abrogate the SAC after nocodazole treatment, in concert with the p38 MAPK [52]. The regulatory CK2β subunit also appears to have mitotic roles that are independent of the catalytic CK2 subunits [53]. CK2β interacts with and increases the catalytic activity of Checkpoint Kinase 1 (Chk1), independently of the CK2 catalytic subunits [54]. Through its interaction with Chk1, CK2β has been demonstrated to control the degradation of the cell cycle regulatory CDC25A phosphatase [55]. Furthermore, knockdown of CK2β leads to stabilization of Wee1, promoting increased phosphorylation of Cdk1 that leads to inhibition of the onset of mitosis [53]. Large-scale RNAi (RNA interference) screens have also highlighted a role for CK2 in cell division. For example, a screen in *Drosophila melanogaster* showed that knockdown of the *CkIIα* gene led to mitotic abnormalities, including centrosomal defects and lagging chromatids upon separation in anaphase [56]. As in the G1/S DNA damage checkpoint, CK2 plays a role in p53-mediated DNA damage signalling in G2. While many proteins instrumental to the G2/M DNA damage checkpoint are substrates and/or interactors of CK2, including the checkpoint kinases Chk1 [54] and Chk2 [57], Topoisomerase II [58, 59], BRCA1 [60], and Plk1 [61], the role of CK2 in execution of the G2/M DNA damage response remains unknown.

Many cell cycle regulatory proteins are phosphorylated by CK2, including Topoisomerase II [58, 59], Cdc34 [62], Cdk1 [63], and Six1 [64]; however, the precise details of these phosphorylation events remain largely unknown. CK2 phosphorylation of Topoisomerase II was initially detected in mammalian cells using the mitosis-specific MPM-2 (mitosis protein monoclonal-2) and 3F3/2 phosphospecific antibodies, which recognize the CK2 sites at serine 1469 and threonine 1342, respectively [58, 59]. Intriguingly, CK2 can regenerate a number of other MPM-2 reactive phosphoepitopes lost following phosphatase treatment, indicating that there are additional mitotic substrates of CK2, and that the MPM-2 antibody may be instrumental in detecting them [58].

In addition to mitosis-specific substrates, CK2 also forms protein-protein interactions in a mitosis-specific manner, using the four mitotic phosphorylation sites located in the CK2α C-terminal (Fig. 1C). One of the most intriguing CK2 interactors in mitosis is Pin1, a peptidyl-prolyl isomerase which catalyzes the cis-trans isomerization of proline residues adjacent to phosphorylated serine or threonine [65]. Due to the similarities between its isomerization consensus sequence and the phosphorylation consensus sequence of Cdk1, Pin1 binds to several important mitotic phosphoproteins, and is believed to have a central role in the regulation of mitosis (reviewed in [66]). Studies in our laboratory have shown that Pin1 binds specifically to CK2α on its phosphorylated C-terminal tail. Furthermore, this interaction leads to decreased CK2-catalyzed phosphorylation of Topoisomerase IIα [67]. It is currently unknown whether Pin1 merely binds to the CK2α mitotic phosphorylation sites or actually catalyzes isomerization of the adjacent prolines, but what is clear is that protein-protein interactions between Pin1 and phosphorylated CK2α can regulate CK2 activity. Recently, a proteomic screen of Plk1 Polo-Box Domain (PBD) interactors in mitosis identified CK2α as a cell cycle-specific, phosphorylation-specific interactor of Plk1 [61]. Intriguingly, threonine 344, one of the four mitotic phosphorylation sites located on the CK2α C terminal, fits the consensus sequence for PBD binding [68]. Plk1 is an important kinase regulating multiple facets of mitotic progression (reviewed in[69]), and determining the role of this interaction in mitotic progression could shed new light on the role of CK2 in mitosis. As noted earlier, CK2β is also phosphorylated by Cdk1 in mitosis, but phosphorylation-dependent interactions have not yet been identified (Fig. 2C).

The emergence of proteomic studies of mitotic phosphorylation and interaction events have yielded some new targets for CK2. Proteomic investigations have confirmed previous localization studies by identifying CK2 as a component of both the centrosomes [70] and spindle midbody [71]. Phosphopro-

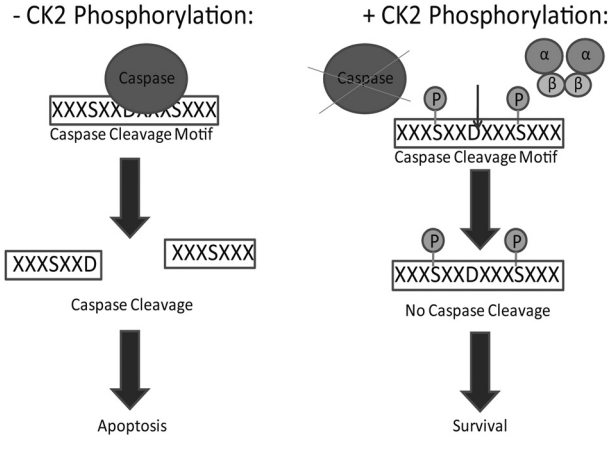

Figure 2. CK2 phosphorylation of caspase substrates can rescue from caspase cleavage. In the absence of CK2 phosphorylation, caspases are free to access the aspartic acid cleavage site and cleave substrate proteins, leading to apoptosis. However, CK2 phosphorylation of adjacent serine (or threonine) residues effectively block caspase binding to a substrate, blocking cleavage and allowing for cell survival.

teomic screens have identified additional proteins presumed to be mitotic substrates of CK2, including Septin-2, INCENP, and MAP7 [72]. While proteomics has identified novel candidate proteins that may interact with CK2 in mitosis, to date proteomics approaches to identify phosphorylation-specific targets and interactors of CK2α have had limited success. This may be at least in part due to the sequence of the CK2α C-terminal tail, as a lack of lysine residues leaves the C-terminal fragment too large to ionize efficiently after typical trypsin digestion. For this reason, large mitotic proteomic screens have been of limited value to the study of CK2α phosphorylation. How CK2 affects mitotic progression, and particularly how this is affected by phosphorylation of the CK2α and CK2β subunits, will be crucial to understanding cell cycle regulation in mammalian cells.

CK2 in the induction of apoptosis

It has long been known that CK2 has an important survival role in mammalian cells. CK2 is required for viability in genetically tractable organisms such as *Saccharomyces cerevisiae* [73], *Schizosaccharomyces pombe* [74], and *Dictystelium discoideum* [75]. Expression of kinase-inactive CK2 in yeast does not rescue the loss of viability, indicating that CK2 catalytic activity, and not simply the presence of CK2, is required for viability [21, 76]. In mouse knockout models, knockout of the regulatory CK2β gene is lethal, even at the single-cell level [77]. Interestingly, while knockout mice lacking expression of CK2α are embryonic lethal at day 10.5 [78], CK2α′ knockout mice are viable, albeit showing defects in spermatogenesis due to a predisposition to apoptosis in the germ cells [79]. Taken together, this indicates that CK2α can compensate for the loss of CK2α′ in most cases, but CK2α′ cannot compensate for loss of CK2α. Interestingly, overexpression of a kinase-dead mutant of CK2α′ in human cancer cells leads to loss of viability [80], indicating that CK2α′ may have a specialized role in maintaining viability not seen with CK2α. Studies using RNAi strategies to knockdown CK2 subunits have had varying degrees of success; however, some have shown that loss of CK2 in this manner leads to decreases in viability [81, 82].

Recently, the role of CK2 in the decision processes leading to apoptosis has become an exciting area of research, as the evidence that CK2 is an important prosurvival enzyme increases. Overexpression of CK2 is protective against drug-induced apoptosis [83], and cell lines that show resistance to apoptosis-inducing drugs often overexpress CK2 [84]. CK2 is thought to directly regulate both receptor-mediated apoptosis [85] and intracellular apoptosis initiated by DNA damage [86]. Additionally, inhibition of CK2 can sensitize cancer cells against both types of apoptosis [87, 88], indicating that chemical inhibition of CK2 may be a promising cancer therapeutic. Interestingly, chemical inhibition of CK2 also enhances the ability of natural killer (NK) cells to kill cancer cells *in vivo* [89]. Many proteins involved in apoptotic signalling are direct substrates of CK2, while others are affected at the level of expression. Survivin, an inhibitor of apoptosis protein (IAP), is upregulated by increased CK2 expression [90]. In contrast, Bid, a pro-apoptotic member of the Bcl-2 family of proteins, binds tightly to and is phosphorylated by the CK2α subunit in particular [91]. Phosphorylated Bid is less susceptible to Caspase 8 cleavage, inhibiting Bid-mediated activation of the mitochondrial apoptotic machinery [92]. This particular form of regulation has increasingly been observed, and may represent a general anti-apoptotic role for CK2, in which phosphorylation by CK2 leads to protection from caspase cleavage [8]. By phosphorylating proteins at residues close to or contained within caspase cleavage sites, proteins are protected from caspase cleavage and apoptosis is avoided [93]. Because of the similarities between the CK2 consensus sequence for phosphorylation (S/T-X-X-Acidic) and the consensus sequences for caspase cleavage, which center around an acidic aspartic acid residue, CK2 is well suited to act in this manner [8] (Fig. 2). Other caspase substrates regulated in this manner by CK2 include Max [94], haematopoietic lineage cell-specific protein 1(HS-1) [95], Presenilin-2 [96], Connexin 45.6 [97], and PTEN [98]. In addition to regulating caspase activity by phosphorylating substrates, CK2 can also directly regulate the activity of the caspases themselves. In mice, caspase 9 is protected from caspase 8 cleavage by CK2 phosphorylation, and loss of the phosphorylation site leads to induction of apoptosis [99]. By phosphorylating caspase 2, CK2 inhibits caspase 2 dimerization, therefore preventing its activation [100]. The caspase-inhibiting protein ARC (apoptosis repressor with caspase recruitment domain) requires phosphorylation by CK2 to effectively inhibit caspase 8 activation [101]. Collectively, it is clear that CK2 has multiple, overlapping roles in induction of apoptosis, which almost certainly contribute to its importance in cellular signalling and oncogenic activity.

Transcriptional control by CK2

Cellular decisions to grow and survive are often mediated through control of transcription, and CK2 is emerging as an important regulator of transcription.

Not surprisingly, the effects of CK2 activity on the transcription of tRNA, rRNA, and mRNA involve multiple levels of regulation and a plethora of substrates. At the level of the basal components of the transcriptional machinery, CK2 can directly regulate the activity of human RNA polymerases I [102, 103], II [104], and III [105]. Despite the varying mechanisms used to initiate and continue transcription by each of these polymerases, the role of CK2 in each system is remarkably similar, and suggests that CK2 may act as a general regulator of all cellular transcription. Transcriptional activation is regulated at the general level in response to cell stimuli, in that cells receiving proliferative signals increase transcription, whereas cells which are subjected to metabolic stresses or DNA damage decrease transcription. As a kinase involved in signalling pathways governing cell growth and survival and directly involved in stress signalling, CK2 is well-placed to be the 'messenger' that relays the current condition of the cell to the transcriptional machinery.

CK2 copurifies with RNA Polymerase I (RNAP I), which transcribes ribosomal RNA (rRNA) subunits, and is present at rRNA promoters [102, 103, 106]. In particular, this interaction takes place between CK2 and the initiation-competent RNAP Iβ, and targets CK2 activity towards the transcriptional activator UBF (upstream binding factor), selectivity factor 1 (SF1) subunit TAF_I100, and RNAP Iβ-associated Topoisomerase IIα [102]. Phosphorylation of UBF by CK2 stabilizes its association with SL1 at the promoter, leading to increased transcription [102, 103], while phosphorylation of TAF_I100 decreases the association of SL1 and UBF at the promoter, leading to decreased transcription. CK2 inhibition limits transcription to one round, indicating a role for CK2 in reinitiation of transcription [102]. In addition, CK2 phosphorylates transcription initiation factor TIF-IA, releasing it from RNAP I. This release may be required for transcriptional elongation. TIF-IA reassociates with RNAP I after dephosphorylation by FCP1 (TFIIF-associating CTD phosphatase), suggesting that interplay between CK2 phosphorylation and FCP1 dephosphorylation is necessary for multiple rounds of rRNA transcription by RNAP I [107].

RNA Polymerase III (RNAP III), which transcribes transfer RNA (tRNA) as well as some rRNA components, is also directly regulated by CK2. CK2 binds to and activates the TBP subunit of the RNAP III general transcription factor TFIIIB [108]. Chemical and peptide inhibitors of CK2, as well as antisense oligonucleotides and expression of kinase-dead mutants of CK2, can block RNAP III transcription in human cell extracts, and this is because CK2 inhibition compromises the ability of TFIIIB to bind another RNAP III general transcription factor, TFIIIC2, which normally recruits TFIIIB to the promoter [105]. CK2 is a required component of reconstituted minimal RNAP III complexes. While CK2 phosphorylation of TFIIIB is required for RNAP III transcription, CK2 can also inhibit RNAP III transcription, through the phosphorylation of additional TFIIIB subunits Brf1 and Bdp1 [109]. Interestingly, the different effects of CK2 on RNAP III transcription seem to be cell cycle-specific. CK2 phosphorylates Bdf1 in mitosis, leading to decreased RNAP III transcription, and inhibition of CK2 in mitosis restores transcriptional activity, while inhibition of CK2 in S phase inhibits transcription [110]. These results show that CK2 is a critical component of RNAP III-mediated transcription, and has both positive and negative regulatory roles on the transcriptional machinery.

The most well studied transcriptional machinery involves RNA Polymerase II, responsible for transcribing mRNA, and the role of CK2 in regulating RNAP II transcription displays some overlap with its regulation of RNAP I. CK2 phosphorylates RNAP II on the C terminal domain (CTD) of the largest subunit [104, 111]. As with phosphorylation of TIF-IA in the context of RNAP I transcription, this phosphorylation event is reversible and involves dephosphorylation of the CTD by FCP1. This cycle of phosphorylation and dephosphorylation is required for the recycling of RNAP II and reinitiation of transcription. CK2 also phosphorylates FCP1 itself, resulting in stimulation of FCP1 phosphatase activity and enhanced binding of FCP1 to TFIIF [112]. In this way, CK2 regulates both RNAP II directly as well as FCP1, the reciprocal phosphatase required for removal of CK2-added phosphates. In addition to phosphorylation of the RNAP II CTD, CK2 also phosphorylates a variety of RNAP II general transcription factors, including TFIIA, TFIIE, and TFIIF. The result of these phosphorylation events is increased formation of initiation complexes at the promoters of genes and increased transcript formation [104].

Extending past the effects of CK2 regulation on the general transcriptional machinery, several transcription factors are themselves substrates of CK2. In fact, of the 307 putative substrates listed by Meggio and Pinna in 2003, 60 were transcription factors [113]. Transcription factors regulated by CK2 phosphorylation and/or interaction include NFκB (nuclear factor-kappa B) [114], STAT1 (signal transducer and activator of transcription 1) [115], CREB (cAMP-response element binding protein) [116], IRF-1 and -2 (interferon regulatory factor-1 and -2) [117], ATF1 (activating transcription factor 1) [116], SRF (serum response factor) [118], Max [119], and the protooncogenes c-

Jun [116, 120], c-Fos [116], c-Myc [119], and c-Myb [119]. The end results of these phosphorylation events can involve activation or repression of transcription, depending on the factor. Due to its direct and indirect effects on transcription, CK2 gains another level of control over pathways invoking many cellular processes, including proliferation and death, by regulating the cell at the level of transcription.

CK2 in viral infection

Infection with various viral agents can lead to deregulation of a cell's normal growth and survival pathways, often leading to disease. An intriguing consequence of the seemingly constitutive activity of CK2 is that several viruses have evolved to use it as a source of phosphorylation for various proteins involved in the viral life cycle, including Epstein-Barr Virus (EBV) [121–123], Herpes Simplex Viruses (HSV) [124, 125], Hepatitis B and C Viruses (HBV, HCV) [126–128], Human Immunodeficiency Virus (HIV) [129], Human Cytomegalovirus (CMV) [130] and Human Papilloma Virus (HPV) [131]. In fact, in a 2003 review of CK2 substrates, Meggio and Pinna listed over 40 different viral proteins shown to be CK2 substrates [113]. Phosphorylation of viral proteins by CK2 can have many effects, including enhanced nuclear localization [130], modulation of DNA binding [132], regulation of viral enzyme activity [122, 129], targeting of viral proteins for degradation by the 26S proteasome [127], and replication and transcription of the viral genome [133]. For example, HIV-1, the causative agent of Acquired Immunodeficiency Syndrome (AIDS), exploits CK2 to phosphorylate several proteins involved in viral infection and replication. The HIV-1 Rev transactivator is phosphorylated by CK2, an event which seems to downregulate Rev activity [129]. However, the interaction between CK2 and Rev actually stimulates CK2 activity towards a number of other HIV-1-encoded genes, including gp120, reverse transcriptase subunits p66 and p51, gp41, and the p27 and p17 caspid proteins [134]. Phosphorylation by CK2 results in increased enzymatic activity of both the HIV-1 reverse transcriptase and protease, and inhibition of CK2 reverses activation [135, 136]. In a macaque model, mutation of CK2 phosphorylation sites in the SHIV (simian/human immunodeficiency virus) to glycine resulted in decreased T cell loss, indicating that CK2 phosphorylation may contribute to the pathogenicity of HIV-1 [137]. Interestingly, several drugs investigated as HIV inhibitors have been demonstrated to selectively inhibit CK2 [138].

Perhaps one of the best elucidated relationships between CK2 activity and viral infection involves HSV Type 1. Kinase activity detected in preparations of purified HSV-1 virions has been attributed to CK2, with HSV-1 proteins including VP12 and VP23 phosphorylated by CK2 [139]. CK2 also phosphorylates the structural protein VP16 at serine 375, and mutation of this site to alanine abrogates formation of a complex between VP16 and the cellular factors Oct-1 and HCF, abolishing transactivation of viral immediate-early genes [140]. One of these immediate-early genes, ICP27 (also called IE63), is phosphorylated by CK2 at serines 16 and 18 [141], and copurifies with CK2 and heterologous nuclear ribonucleoprotein K (hnRNP K) [125].This complex was later found to include p32, a cellular protein involved in mRNA splicing, which is also phosphorylated by CK2 in the ICP27 complex [142]. Like HIV-1, HSV-1 seems to be capable of modulating CK2 activity, as HSV-1 infection stimulates CK2 activity early after viral infection, relocates the CK2 holoenzyme from the nucleus to the cytoplasm, and targets CK2 towards the phosphorylation of hnRNP K, a protein not normally phosphorylated by CK2. These changes are dependent on ICP27 expression, and are believed to facilitate the HSV-1 lytic cycle [143]. There are many other examples of viruses which seem to hijack CK2 activity to ensure their own survival. The involvement of CK2 in the life cycles of these viruses suggests that it may be a useful target for antiviral drugs, however in order to successfully achieve this, much more needs to be known about the specific regulatory events acting on CK2.

CK2: A 'regulatory linker' between cellular processes?

One of the confounding factors of the study of CK2 lies in its multiple roles and plethora of substrates involved in a variety of cellular processes. The major roadblock in understanding its function (and consequently, its promise as a therapeutic target) lies in determining which functions are essential and which are more complementary, and reconciling the frequently conflicting functions of CK2 and how these various signals are interpreted by the cell to lead to the appropriate response. For example, CK2 is required for viability, as pharmacological inhibition, RNAi, and overexpression of catalytically-inactive CK2 subunits all lead to decreased viability [80–82, 87, 88]. However, it remains unknown exactly which functions of CK2 are required to maintain viability. Is it through proper cell cycle regulation, through prosurvival signalling, or possibly through transcriptional control? Similarly, while CK2 is overexpressed in many

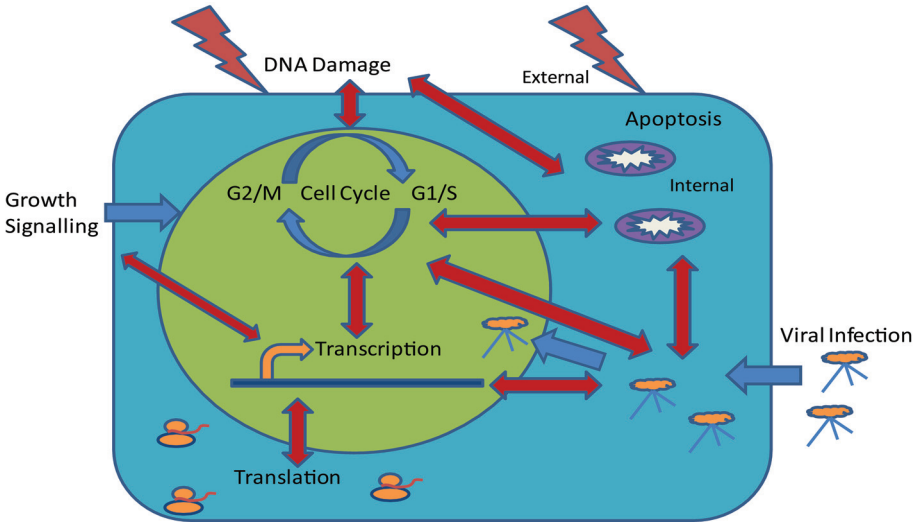

Figure 3. CK2 as a lateral signal between cellular processes. Overview of pathways involving CK2 activity that are described in the text. Red arrows signify links between cellular processes which can be communicated through various CK2 functions.

forms of human cancer [8], it is unknown which functions of CK2 are altered by its increased expression, leading to oncogenesis. An alternative explanation to the existence of one main role for CK2 is that deregulation of the proper interplay between these functions may be the cause of defects seen with aberrant CK2 expression. In the human genome, CK2 consensus sequences are abundant, and phosphorylation by CK2 has been estimated to represent up to one quarter of the eukaryotic phosphoproteome [113]. In evolutionary terms, most proteins have developed very specific, tightly regulated functions, including numerous other protein kinases. The very existence of a protein kinase with ubiquitous expression, constitutive activity, and high number of substrates points to there being a specific reason for its existence. It has been suggested that unlike the linear, vertical role that most protein kinases play in signal transduction pathways, CK2 may display a more 'lateral' means of pathway intervention, allowing for a global level of control which may serve to consolidate the actions of different pathways [113] (Fig. 3). If CK2 is to be viewed as a master regulator of cellular functions, its overexpression in cancer and oncogenic properties may be due solely to a cellular advantage gained from neutralizing control over which CK2 activities are heeded and which are ignored in response to other, more urgent responses. There are several examples of crosstalk between seemingly distinct CK2 functions. Through its involvement in cell cycle progression and apoptosis, CK2 has emerged as a possible link between the two processes, and may mediate cellular decisions to either continue proliferation or self-destruct. The roles of CK2 in the cell cycle, particularly in G1/S, typically involve DNA damage checkpoint signalling, including its effects on p53 transcriptional activation. Not only could CK2 accentuate damage signals through modulating p53 activity, but as an enzyme intricately and directly involved in the induction of apoptosis, it may act both as the stimulus and the effector in the pathways determining the decision to die. In this effect, the relationship between p53 and CK2 could be crucial for proper monitoring of genomic integrity in proliferating cells. While p53 is frequently mutated or otherwise functionally inactive in cancer (reviewed in [144]), CK2 is often overexpressed (reviewed in [8]), leading to twofold deregulation of these signalling pathways. Perhaps not surprisingly, CK2 overexpression combined with loss of p53 acts synergistically in the development of thymic lymphomas in mice [145]. This link between cell cycle control and induction of apoptosis may also occur during mitosis. Recent work in our laboratory has shown that by disrupting the temporal regulation of CK2α phosphorylation during mitosis, cells can be stopped from dividing by induction of mitotic catastrophe (St-Denis et al., submitted manuscript), a type of mitotic cell death involving activation of the apoptotic machinery [146]. Presumably, this shift would involve a decrease in mitotic signalling and an increase in apoptotic signalling. How this switch actually occurs is unknown, and this information is crucial to our understanding of CK2 regulation and induction of mitotic catastrophe in general.

Recently, the traditional view of cell cycle progression has been challenged [147], with the notion that transcriptional oscillation throughout the cell cycle may serve to cause cell cycle progression even in the absence of activating Cdks [148]. In some respects this is not surprising, as transcription has long been known to be modulated thoughout the cell cycle. Indeed, some proteins crucial for progression through the cell cycle also have roles in transcriptional control, such as CAK, which phosphorylates RNA Polymerase II and

various transcription factors as well as Cdks [26]. Similarly, CK2 may very well act as a linker between cell cycle control and transcription, given its many functions in both areas.

The exploitation of CK2 activity by viruses can have far-reaching effects on CK2 regulation, and represents another example of how CK2 can act as a lateral signalling molecule between different cellular functions. Viruses have been shown to stimulate the activity of CK2 [134, 143]. In particular, stimulation of CK2 leading to increased RNAP II CTD phosphorylation can lead to increased transcription of viral genomes [149]. Viruses that are mutated to no longer activate CK2 show decreased proliferation and apoptosis, suggesting that viruses may enhance the anti-apoptotic and proliferative roles of CK2 [143].

Conclusion

Through the work of multiple groups into multiple aspects of CK2 activity, it is clear that the proper regulation of CK2 is crucial throughout the life cycle of the mammalian cell, from cell division to produce a nascent daughter cell, throughout the life of that cell, and ultimately in the decision to undergo apoptosis. In addition to direct effects on the pathways controlling these functions, CK2 also influences the life cycle of the cell through regulation of transcription. In the event of viral infection, CK2 activity is adopted to enable viral infection and replication, altering the normal life cycle of the cell. The plethora of research into the various cellular roles of CK2 has also shed light on the intricate regulatory mechanisms controlling phosphorylation of its substrates. While much needs to be learned before the underlying function of CK2 in the cell is completely understood, we suggest that CK2 may act as a regulatory node which coordinates various signals into an appropriate cellular response. Increased understanding of the actions of CK2 and how they are regulated will aid in evaluating the prospect of exploiting CK2 as a therapeutic target.

Note added in Proof: Reference 150 has been added during the proof procedure.

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