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Protein Kinase CK2

A Global Regulator of Cell Behavior

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Summary

Cells are highly dynamic entities whose behavior is constantly shaped by the numerous physiological and physical stimuli they perceive. This plasticity allows cells to survive and adapt to stressing environments, or to alter their fate in response to developmental cues. Signal transduction pathways evolved as a way to connect the reception of stimuli by cellular sensors to effector molecules that can change the behavior of the cell. The integration of different stimuli that allows for a directed and coordinated cellular response relies on extensive cross-regulation and overlap between distinct signaling pathways through processes that remain poorly understood. To be effective, signal transduction pathways need to be swiftly activated when exposed to specific stimuli and inactivated when stimuli are absent. Reversible protein phosphorylation can act as a molecular switch, drastically altering protein behavior, and has emerged as a prominent regulatory mechanism in eukaryotic signaling pathways. Protein kinases are the catalysts of protein phosphorylation in cells and their activity is usually subject to precise spatiotemporal regulation. The essential and conserved protein kinase CK2 (CK2), however, lacks this typical regulation and its precise cellular functions remain mysterious despite extensive research. CK2 is present is all eukaryotic cells and most cellular compartments and, even though it is involved in the transduction of numerous stimuli, CK2 is constitutively active. Attesting to its importance, changes in CK2 cellular dynamics are usually associated with significant changes in cell behavior in many eukaryotic species. Possibly the best characterized function for CK2 is its activity as a potent and multifunctional promoter of cell growth and survival. For this reason CK2 is currently considered a promising target for cancer therapy. Nonetheless, CK2 activity has been associated with countless other cellular processes, including cell cycle progression, differentiation, cell migration, polarity establishment, and transformation. In this review, I will highlight significant developments in the study of the mechanisms regulating CK2 activity, as well as the contribution of CK2 to the regulation of innumerous proteins involved in cellular signaling and in the most fundamental mechanisms that characterize eukaryotic cells. I will also discuss the putative function of CK2 as a master regulator of cell fate, whose activity is dynamically defined by the environment and necessary to coordinate the permanent process of cellular adaptation. Ultimately, the continued effort to understand the function of CK2 in a global context promises to convey many insights into how cellular processes are integrated and how cells behave in response to their environment.

Abstract

Cellular adaptation to the constant changes in internal and environmental conditions is a key strategy for cell survival and development. In eukaryotes, cellular adaptation generally depends on the regulated activation of well-defined and condition-specific molecular signaling cascades that activate terminal effectors responsible for altering the cell behavior. In contrast, the activity of the atypically regulated protein kinase CK2 has been puzzling researchers for decades and a concrete model of its function in cells remains to be established. The highly pleiotropic CK2 is conserved and ubiquitously expressed in all eukaryotic cells, localizes to virtually all subcellular structures, and is constitutively active irrespective of the cellular conditions. Nevertheless, distinct cellular conditions are known to differentially modulate the catalytic activity, subcellular localization, and substrate preference of CK2, eliciting specific CK2-driven cellular responses. Classically, CK2 has been associated with growth promotion since it acts at multiple levels to induce growth signaling pathways and suppress apoptosis. Mounting evidence suggests that CK2 directly regulates an even wider range of signal transduction pathways in development and stress response, as well as most of the conserved eukaryotic mechanisms required for gene expression, growth, and cellular integrity. In this review, I will focus on discussing relevant information concerning the regulation of CK2 and its activities in signal transduction and regulation of vital cellular mechanisms. I will further discuss how CK2 can act as a crucial factor in determining cellular behavior and adaptation by integrating numerous stimuli, coordinating diverse signaling pathways, and directly modulating the activity of cellular mechanisms.

Introduction

Development and survival of cells depends on their ability to perceive and efficiently respond to the ever-changing intra- and extracellular environments. The cell's capacity to undergo a directed and sustained response is remarkable, given the constant, diverse, and often contradictory nature of the stimuli it receives. To achieve such a response, the plethora of stimuli sensed by cellular receptors/sensors must be interpreted and integrated before engaging the cellular machinery responsible for functional and morphological change. Intuitively, this implies that stimuli integration depends on a global crosstalk between different pathways, and that signaling from these integration points is tightly regulated to ensure a coordinated cellular response. Indeed, decades of research and recent 'omic' approaches have revealed numerous components and regulatory mechanisms involved in signal transduction. Notably, this collective research has unveiled an extensive convergence and connectivity between signaling pathways that underlie stimuli integration and the subsequent coordinated response.

In eukaryotes, reversible protein phosphorylation emerged as a prominent regulatory mechanism in all stages of signal transduction and for determining the activity of basal cellular processes. The phosphorylation status of a protein depends on the dynamic interplay between protein kinases and phosphatases. Phosphorylation can greatly influence the behavior of a target protein including its intrinsic function, half-life, localization, and interactions with other molecules. Increasing levels of complexity are achieved by combination of these events in the same protein via multisite phosphorylation [1]. Importantly, the dynamics created by multisite phosphorylation are the key for stimuli integration and can modulate the degree and duration of a response [2, 3]. In accordance with the dynamic behavior required for signal transduction, misregulation of protein kinases and mutations that confer constitutive/inactive kinase function are often associated with altered developmental programs and disease progression. The biological impact and regulatory potential of kinase-mediated phosphorylation is further attested by the evolutionary conservation of the eukaryotic protein kinase superfamily, and radiation of protein kinase families at increasing levels of eukaryotic complexity [4].

Under physiological conditions, most protein kinases are either not expressed or kept in an inactive state, being activated by other molecules following specific stimuli. At odds with this typical dynamic regulation, the highly conserved protein kinase CK2 (CK2) is ubiquitously expressed and constitutively active in eukaryotic cells. Nevertheless, this atypically regulated kinase appears to play a pivotal and rather unique role in signal transduction and control of basal cellular processes, both under physiological conditions and in stress/stimuli responses. CK2 was discovered along with CK1 in 1954 by Burnett and Kennedy, and their activity provided the first example of an enzymatic activity that phosphorylates a protein. Since then hundreds of putative CK2 substrates and an ever-growing list of physiological targets and binding partners

have been described [5, 6]. CK2 is present at basal levels in all eukaryotic cells, in many subcellular compartments and structures, and in the extracellular matrix (ECM) as an ectokinase. Even though regulation of CK2 remains obscure, cell-autonomous changes in its expression, activity, subcellular localization, and target specificity have been documented during development, and in response to numerous intra- and extracellular stresses. Classically, CK2 has been labeled the most promiscuous eukaryotic kinase, a claim now supported by proteomic studies showing that most human phosphosites are contained within a CK2 consensus phosphorylation sequence [7, 8]. Along with the multitude of substrates come descriptions of CK2-dependent regulation of a myriad of cellular processes, commonly associated with signal transduction and 'housekeeping' regulatory functions [9]. These include processes that are precisely regulated, for example cell division, or 'constitutive' such as the circadian clock. In contrast with the usual hierarchical *modus operandi* of kinases in signaling cascades, CK2 is involved in numerous signaling pathways and often at multiple stages of signal processing. Notably, CK2 also appears to be a multilevel regulator of gene expression and overall protein activity through phosphorylation of targets associated with chromatin remodeling, transcription, splicing, translation, and protein maturation and transport. As expected from this pleiotropy, disruption of CK2 activity by genetic, chemical, or RNA interference assays confers lethality in all species tested, suggesting that CK2 activity is essential for eukaryotic life.

The interest in this enzyme has recently increased with mounting evidence that CK2 plays an important role in promoting growth, proliferation, and suppression of apoptosis in normal and transformed cells. Elevated CK2 activity is found in yeast cells with higher growth rates, in proliferating mammalian cells, and after hormone and growth factor stimulation compared with unstimulated controls. Importantly, higher levels of CK2 activity are also detected in most cancers and cancer cell lines, which is positively associated with a poor prognosis. Although CK2 cannot be strictly termed an oncogene, its activity seems to create a more favorable environment for cell survival and proliferation. CK2 activity shows cooperativity with many oncogenes and CK2 overexpression induces development of lymphomas and breast tumors in mice [10]. Interestingly, pharmacological or biological interference with CK2 activity dose-dependently reduces tumor growth and leads to induction of apoptosis in human cell lines and animal models [11, 12]. Indeed, accumulating evidence argues for an extensive role of CK2 in the suppression of the apoptotic cascade [13]. With this dual role of both promoting survival and inhibiting cell death, CK2 is now seen as a promising candidate for prognostic biomarker development and therapeutic target in cancer treatment.

Despite extensive research across model organisms and hundreds of publications that demonstrate the incredible pleiotropy of CK2, its cellular regulation and function remain elusive, and few discussions consider more than a few specific facets of CK2 activity. The paradoxical conjugation of a ubiquitous and constitutively active enzyme with the spatiotemporal regulation of numerous fundamental processes

challenges current views of whole-cell behavior and is poorly understood. Here, I will review relevant findings that illustrate the importance and pleiotropy of CK2 function in eukaryotic life, and discuss its putative function as a global coordinator of cell behavior through the dual role of permanently sensing the state of the cell, and specifically modulating the activity of signaling pathways and cellular processes to promote cellular adaptation to different environmental conditions.

The puzzling properties of an essential protein

The CK2 holoenzyme is a tetramer invariantly composed of two catalytic (CK2α) and two regulatory (CK2β) subunits, although species differ slightly in the number of genome encoded α and β subunits. Mammalian genomes code two distinct catalytic subunits CK2α and CK2α' and a single regulatory CK2β protein that form different CK2 holoenzyme configurations: CK2ααββ, CK2αα'ββ, and CK2α'α'ββ. CK2α (and α') is generally described as a constitutively active kinase since no gain-of-function mutants have been isolated, and since neither post-translational modifications nor interacting molecules are necessary to switch CK2α into an active kinase. Instead of the usual on/off switch characteristic of kinase regulatory subunits, CK2β regulates CK2α/α' by modulation of target specificity, target-specific catalytic activity, subcellular localization, and holoenzyme stability. Contributing to the singularity of this enzyme, CK2α/α' is a dual-specificity kinase that phosphorylates both Serine/Threonine (Ser/Thr) and Tyrosine (Tyr) residues in acidic motifs [14] Moreover, CK2 is equally efficient at using GTP and ATP for phosphate group transfer. The minimum consensus sequence for CK2 phosphorylation requires an acidic residue at position n+3 downstream of the phosphoacceptor site (S/T-x-x-D/E). A preference for additional acidic residues and absence of basic residues is generally observed in the proximity of the target site [5, 8]. Interestingly, CK2α/α' can recognize phospho-Ser and phospho-Tyr residues as the critical n+3 acidic residue, indicating that this kinase is likely to participate in hierarchical phosphorylation events [15, 16]. This type of regulation prevents untimely or unlicensed signaling from CK2α and may be an important cellular mechanism to control its constitutive activity.

The two mammalian catalytic subunits share 90% sequence similarity, with the main differences located at the unstructured C-termini that is highly conserved between species. This divergence may underlie the distinct expression patterns and binding partners of the two CK2 catalytic subunits. In spite of their dissimilarities, mammalian CK2 kinases share some degree of functional conservation since CK2α can partially compensate CK2α' absence in homozygous mice [17]. Further confirmation of CK2 complex activity and regulation comes from studies showing that CK2 subunits have holoenzyme-independent functions in a physiological context. All subunits are ubiquitously expressed in cells, although differences in relative expression can be measured in different cell types and conditions. Within eukaryotic cells, CK2 is found in

distinct compartments including the plasma membrane, cytoplasm, mitochondria, ER, nucleus, and most nuclear structures. While this seems to complicate even further the already complex task of precisely regulating a ubiquitous and constitutive kinase, it is in accordance with its reported pleiotropy. However, dynamic relocalization of CK2 subunits between specific compartments has been frequently observed during development and stress responses, as will be discussed further. This dynamic relocalization upon activation of signaling pathways may be key in allowing a ubiquitous and constitutively active molecule to have distinct and regulated activities.

In addition, both CK2α kinases and CK2β are regulated by phosphorylation and ubiquitination, and CK2α undergoes O-Linked glycosylation [18]. In most cases, the agents and consequences of these post-translational modifications (PTMs) in CK2 activity are unknown. In mammalian cells, CK2 activity induces expression of both CK2α and CK2β, and leads to autophosphorylation of the β subunit protecting it from proteasomal degradation [19, 20]. CK2-dependent CK2β stabilization in turn facilitates CK2β dimerization, which is a prerequisite for holoenzyme assembly [21]. This shows that CK2 catalytic activity acts as a self-perpetuating cue by both promoting subunit expression and holoenzyme assembly. Altogether, these results highlight the importance of identifying stimuli-dependent PTMs and binding partners, which dynamically modulate the specificity and subcellular localization of CK2 subunits, to understand its cellular regulation. Despite many uncertainties, some physiological regulators of CK2 have been identified and will be discussed below.

Several lines of evidence suggest that maintenance of CK2 activity is of vital importance for development and viability of all eukaryotic cells. Accordingly, homologous sequences coding for CK2α and β subunits can be found in sequenced genomes from all branches of the eukaryotic radiation. This conservation in very distant species, including primitive eukaryotes, already hints at an important functional role that is under positive selection. By analyzing protein sequence alignments, the idea that conservation of CK2 structure and function is essential for eukaryotic cells becomes even clearer. Human CK2α protein sequence differs from mice and chicken homologues by three and eight (four of which conservative) amino acid substitutions, respectively. Remarkably, if we consider only the first ~350 residues (out of 391), the mammalian and bird CK2α proteins are identical. Moreover, human CK2α shares extensive sequence similarities with fly, nematode, plant, fungi, slime mold, and alveolate homologues (respectively 91%, 81%, 76%, 66%, 70%, and 66% amino acid identities). The closest CK2α relative is cyclin dependent kinase 1 (Cdk1/Cdc2), a master regulator of cell cycle progression, and both belong to the CGMC group of the eukaryotic protein kinase (ePK) superfamily. This ancient group includes some of the most essential and conserved kinase families such as CDK, GSK, MAPK, SRPK and the dual-specificity CLK and DYRK families [22]. Human Cdk1 protein differs from mice and chicken by nine and twenty-six amino acid substitutions, respectively, indicating that

Cdk1 accumulated approximately three times more substitutions than CK2α in the same evolutionary period. Considering the recognized importance of Cdk1, this somewhat crude comparison illustrates the enormous constraints on CK2α sequence evolution, putatively underlying an essential cellular function.

The sequence of the CK2β subunit does not display significant homology with any known kinase regulatory subunit but is extremely conserved between species. The CK2β protein is identical in birds and mammals, and differs by a single conservative substitution between these animal classes and *Xenopus laevis*. As for CK2α, evolutionary constraints on CK2β evolution are readily apparent in a similar comparison with fly, nematode, plant, fungi, slime mold, and alveolate homologs (respectively 89%, 82%, 58%, 60%, 54%, 41% amino acid identities). For both CK2α and CK2β, conservative substitutions make up for a large part of the observed protein sequence differences between distantly related species. Experiments with budding yeast cells further exemplify the functional conservation of CK2, since expression of human, *Drosophila*, or *C. elegans* CK2α cDNA is able to restore yeast viability in the absence of the endogenous protein [23, 24].

High expression levels and a high number of intermolecular interactions are thought to negatively influence the rate of protein evolution. Hence, the high sequence and functional conservation of CK2 might arise as a by-product of a non-essential primitive pleiotropy and poorly regulated expression. Arguing against a non-essential function, genetic studies in many organisms strongly support the idea that eukaryotic life is not possible when expression of CK2 gene products is disrupted. The fission yeast *Schizosaccharomyces pombe* has a single gene coding for each CK2 subunit. Complete deletion of the *cka1* gene (CK2α) is lethal, whereas a *cka1* temperature-sensitive (ts) mutation leads to defective polarity re-establishment after cytokinesis and cell death when shifted to the restrictive temperature [25]. Disruption of *ckb1* (CK2β) results in viable albeit small, non-polarized, and cold-sensitive fission yeast cells, whereas its overexpression inhibits cell growth and cytokinesis while also causing extensive morphological abnormalities [26]. Similarly, the budding yeast *Saccharomyces cerevisiae* has two genes coding for each subunit, is unviable when both CK2α genes (CKA1/CKA2) are knocked out, but viable upon double CK2β (CKB1/CKB2) deletion [24, 27]. Temperature-sensitive CKA1 and CKA2 alleles show loss of polarity and defects in cell cycle progression, respectively [28, 29]. Despite their viability, CKB1 knockouts show defects in salt sensitivity, and CKB2 deletion mutants display only partial adaptation to G2/M checkpoint arrest following DNA damage, indicating a role for CK2B in the promotion of viability during cell cycle arrest [27, 30].

Recent gene disruption studies in the malaria parasite *Plasmodium falciparum* showed that all genome encoded CK2 subunits (PfCK2α, PfCK2β1, and PfCK2β2) are essential for completion of the erythrocytic asexual cycle [31, 32]. In the nematode *Caenorhabditis elegans* a single gene encodes each CK2 subunit. Homozygous mutants for *kin-3* (CK2α) and *kin-10* (CK2β) are embryonic lethal, and siRNA-mediated knockdown leads to partially penetrant embryonic lethality and viable worms with a slow growth phenotype

and morphological abnormalities [33]. Homozygous null alleles of the fruit fly *Drosophila melanogaster* single CK2 catalytic subunit (DmCK2α) are also larval lethal [34, 35]. *Drosophila* has a CK2β gene that when disrupted or ectopically expressed causes lethality. In contrast to most metazoans, *Drosophila* has two CK2β-like genes (DmCK2β' – adult testes; DmCK2βSSL – male specific) whose function has yet to be clarified [36, 37]. Even though all *Drosophila* CK2 regulatory subunits interact with CK2α to form a tetrameric CK2 holoenzyme, CK2β-like genes cannot rescue deletion of CK2β, suggesting specific functions for distinct CK2βs [36, 37].

Not surprisingly, vertebrate viability is also dependent on CK2 activity. Homozygous knockout mice for CK2α and CK2β die *in utero* (at E11 and post-implantation, respectively) with pleiotropic developmental defects [38]. Furthermore, establishment of homozygous CK2β knockout embryonic stem cell lines has failed using both classical or conditional deletion strategies, suggesting that disruption of CK2β results in immediate cell death. CK2α'$^{-/-}$ mice are viable but males are infertile [39], and double CK2α$^{+/-}$ CK2α'$^{-/-}$ mice show a partially penetrant embryonic lethality phenotype not observed in either single, suggesting that CK2α can partially compensate for CK2α' absence.

The recurrent message that stems from the analysis of CK2 molecular properties, evolutionary history, and loss-of-function mutations, is that this enzyme's cellular functions are necessary and irreplaceable for a eukaryotic cell. In the last decades, much progress has been made to elucidate the molecular basis of CK2 essentiality. Some of these advances concern CK2 regulation of signal transduction pathways, basal cellular processes, and cell fate, and will be the focus of the following chapters. I will focus mainly on illustrative examples from primary publications, considering that it would be nearly impossible to convey all the information available on CK2, and the inherent challenges to conclusively establish physiological kinase-substrate-function relationships.

A global player in cellular signaling

Signaling pathways have evolved to answer the cell's need to sense and adapt to different environmental and intracellular conditions. As previously mentioned, the key to success in such behavioral change is coordination of different pathways such that the appropriate response is promoted and the inappropriate ones are inhibited. Meaningful cellular processes such as growth or cell division provide good examples of the required coordination since undergoing these processes under unfavorable conditions (e.g. absence of nutrients or growth factors, genotoxic stress, parasitic infection, etc.) could have disastrous consequences. The positioning of CK2 within the cell's signaling network is unique given that it responds to numerous stimuli, and regulates multiple signaling pathways often in more than one way. This chapter focuses on

illustrating the multilevel regulatory role that CK2 exerts in signal transduction during cellular stress response, morphogenesis, and growth.

Stress response

Cells are inherently vulnerable to a variety of environmentally-induced stresses, as well as to cellular stress that arises due to faulty or overloaded cellular homeostatic processes, and pathogenic infection. Exposure to stress often leads to changes in CK2 activity and localization. Moreover, cells show higher stress vulnerability when CK2 is inhibited, suggesting that this kinase has a function in the cellular response. For example, a swift and temperature-dependent relocalization of cytosolic and nuclear CK2 to speckle-like nuclear structures and to the nuclear matrix occurs following heat-shock of mammalian cells [40, 41]. Similarly, CK2 dynamically relocalizes after exposure to UV light and ionizing radiation (IR), which is accompanied by an increase in CK2 activity when compared to unstressed cells [40–42]. Under hypoxic conditions, CK2 kinase activity increases, and the catalytic subunits are shuttled to the nucleus. On the other hand, CK2β remains in the cytoplasm, but accumulates at the plasma membrane following long-term hypoxia. Expression levels of CK2β increase under hypoxic conditions, but this protein is not required for hypoxia-induced CK2α- and α'-mediated phosphorylation of HDAC1 and HDAC2 and stabilization of the main hypoxia response mediator HIF-1α [43, 44].

As with many aspects of CK2 function, the cellular regulators behind CK2 stress-induced activity and motility remain mostly unidentified. A notable exception seems to be regulation by stress-activated p38-MAPK, an important kinase in the initiation of stress and immune adaptive responses. CK2 catalytic activity increases in a p38-MAPK-dependent manner [45–47]. This has been observed in mammalian cells exposed to the protein synthesis inhibitors arsenite and ansiomycin, the cytokine TNFα, UV irradiation, or osmotic stress [45–47]. Interestingly, CK2 could also be responsible for maintaining p38-MAPK inactive in unstressed cells, through activation of the phosphatase MKP1. In mammalian cells, depletion and overexpression of CK2α revealed a positive correlation with MKP1 expression, and an inverse correlation with the phosphorylation state of p38-MAPK. CK2 phosphorylates MKP1 *in vitro*, and MKP1 phosphorylation by overexpressed CK2α is abolished by alanine substitutions in two putative CK2 phosphosites. Moreover, siRNA against MKP1 inhibits p38-MAPK dephosphorylation induced by CK2α overexpression [48]. Although not conclusive, these data suggest that CK2 directly phosphorylates MKP1 to regulate the activity of the pivotal stress signaling protein p38-MAPK. It remains to be demonstrated if this putative CK2-mediated MKP1 phosphorylation occurs *in vivo* and is modulated by stress stimuli.

Numerous proteins involved in stress and immune response have been described as CK2 substrates (for review see [5, 49]). From this extensive research, CK2 emerged as a potent regulator of pathways controlling survival and apoptotic stress responses, and in the coordination of stress adaptation mechanisms. Examples

that will be discussed include CK2 regulation of the transcription factors NF-κB and p53, promoting survival and apoptotic responses to stress respectively, and CK2 function in proteotoxic and genotoxic stress response machineries.

NF-κB stress response

In mammals, the conserved NF-κB transcription factor family consists of five proteins that interchangeably associate to form homo- or heterodimeric complexes. These complexes play important and distinct roles in the cellular survival response to multiple threats. In non-stimulated cells, NF-κB dimers are kept inactive in the cytoplasm. Upon stress induction, NF-κB dimers are activated and shuttled to the nucleus, where they can promote transcription of numerous stress-response genes. Given their importance, NF-κB activity is tightly regulated, most notably by their inhibitors of the IκB family, and by IκB kinases (IKK) that promote IκB degradation. The regulatory role of CK2 on NF-κB activity was first shown by its function as an IκBα kinase in unstressed and lipopolysaccharide (LPS)-stimulated cells. Several groups demonstrated that CK2 interacts and constitutively phosphorylates several C-terminal residues of IκBα in cultured cells [50–53]. This CK2-dependent constitutive phosphorylation, which mainly targets Ser293, was shown to be necessary for efficient turnover of free IκBα in unstressed cells, since IκBα half-life increased upon CK2 immunodepletion or mutation of CK2 phosphosites [51–53]. Interestingly, studies with the *Drosophila* IkB homolog *cactus* yielded similar results. Cactus is needed for dorso-ventral patterning during *Drosophila* embryogenesis and is a CK2 substrate *in vitro.* The putative CK2 phosphosites in cactus are constitutively phosphorylated *in vivo*. Moreover, phosphorylation of these sites regulates cactus protein levels and stability in *Drosophila* embryos, and consequentially dorso-ventral axis formation [54]. Later, CK2 activity was found to correlate positively with increased NF-κB expression and activity in tumor cell lines [55–57]. Depletion of CK2α or CK2β by siRNA, and overexpression of CK2α or CK2α' kinase-inactive mutants, were shown to prevent IκBα degradation as well as NF-κB nuclear translocation, DNA binding of NF-κB in tumor cell lines and in a tumor xenograph mouse model [55, 57–59]. Furthermore, overexpression of CK2α in mouse fibroblasts was shown to induce increased IκBα turnover and NF-κB transcriptional activity [55]. Taken together these results indicate that CK2 is a physiological regulator of NF-κB activity. Interestingly, NF-κB was shown to be a transcriptional regulator of CK2α, suggesting that a feedforward regulatory loop may sustain a survival response [60].

The aberrant NF-κB activation observed in carcinoma cells lacking the tumor-suppressor pVHL revealed another role for CK2-mediated NF-κB regulation. The putative scaffold protein pVHL is best known for its inhibition of the hypoxia response factor HIF1α. pVHL interacts with CK2 in cultured cells and is a CK2 substrate *in vitro* [61, 62]. It was shown that pVHL enhances CK2 binding to NF-κB agonist CARD9 in renal carcinoma cells, which facilitates CK2 phosphorylation of multiple residues in CARD9 and abolishes CARD9-dependent activation of NF-κB [62]. These results suggest a possible mechanism for CK2-mediated inhibition of NF-κB in basal conditions or during the apoptotic response to stress. It is currently unknown whether the

phosphorylation of pVHL by CK2 has physiological relevance, or whether the pVHL-dependent induction of CK2 activity serves as a general regulatory mechanism for CK2.

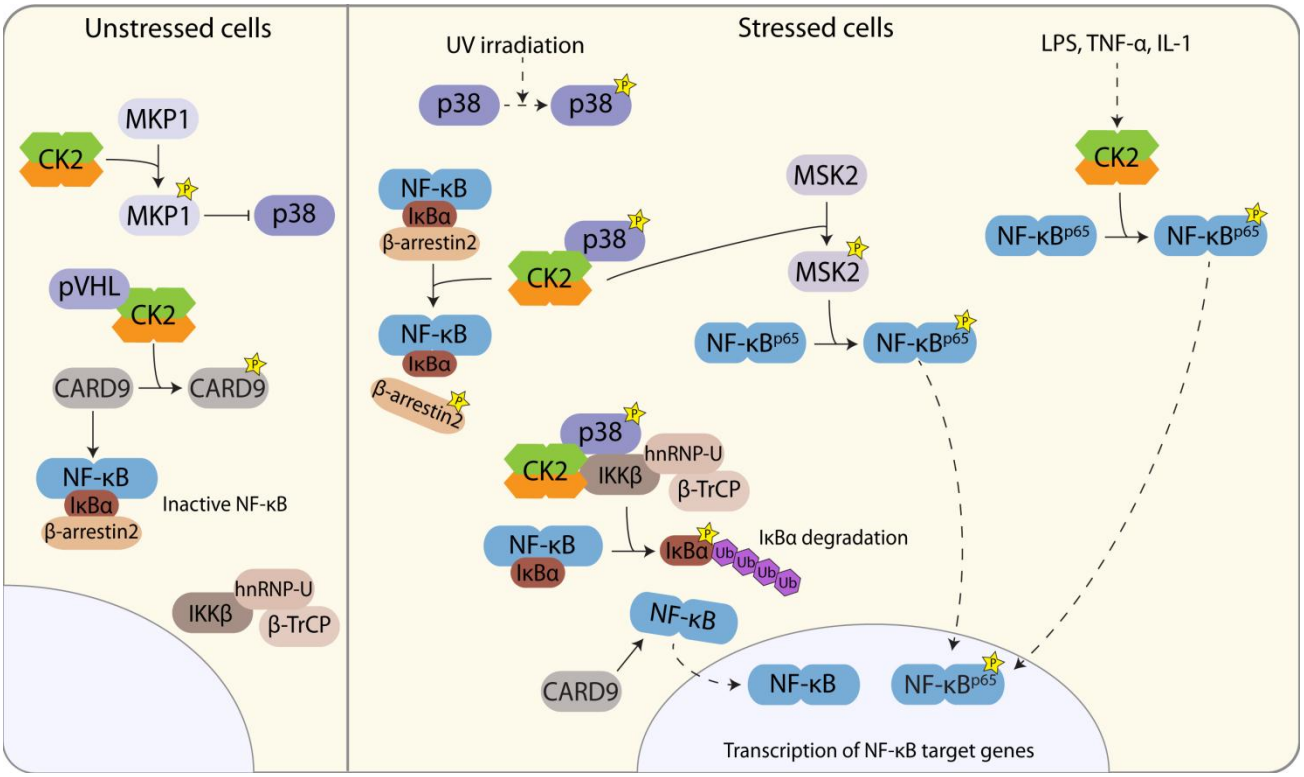

Figure 1 – Schematic representation of CK2 functions in the NF-κB stress response. CK2 inhibits p38 and NF-κB activation in the absence of stress stimuli (left panel), whereas it contributes at multiple levels to the activation of NF-κB when stress is induced (right panel). Solid arrows represent direct regulatory events and broken arrows indicate molecular events that require the activity of additional factors. P – phosphorylation; Ub – ubiquitination.

Regulation of NF-κB activity in the UV response by is one of the best described functions of CK2. Following UV irradiation, NF-κB is activated by a mechanism that requires IκBα degradation and is independent of classical IKK pathways. CK2 quickly binds and phosphorylates IκBα in cells after UV irradiation. CK2β depletion by siRNA abolishes UV-induced IκBα phosphorylation and degradation [46]. Studies with an IκBα mutant resistant to CK2 phosphorylation show that CK2 activity is necessary for rapid turnover of IκBα in UV-irradiated cells. Moreover, CK2-dependent IκBα phosphorylation increases NF-κB DNA binding activity and cellular viability after UV irradiation [46]. UV-induced CK2 activity is abrogated in fibroblasts derived from p38-MAPK$^{-/-}$ mice. In these knockout cells, IκBα degradation and NF-κB DNA binding activity are defective after UV exposure [46, 63]. These results suggest that p38-MAPK is an important regulator of the CK2-dependent activation of NF-κB that confers protection from UV-induced cell death. Subsequently it was found that UV-induced association between the β-TrCP-hnRNP-U-IKKβ protein complex, CK2, and activated p38-MAPK is necessary for efficient IκBα degradation. The scaffold protein IKKβ is constitutively associated with the substrate-recognition subunit β-TrCP of the E3 ubiquitin ligase SCF complex through the ribonucleoprotein hnRNP-U. UV exposure induces the binding of IκBα to IKKβ, and this interaction is

required for UV-induced IκBα degradation. Moreover, UV irradiation promotes IKKβ-dependent recruitment of CK2 and activated p38-MAPK to the β-TrCP-hnRNP-U-IKKβ-IκBα complex, which together with β-TrCP mediate inhibitory IκBα phosphorylation and ubiquitination [63]. The crucial role of CK2 in this pathway is further illustrated by its regulation of an NF-κB suppressor β-arrestin2. β-arrestin2 binds to IκBα and promotes its stability in unstimulated cells. UV-induced CK2 phosphorylation of β-arrestin2 inhibits IκBα-β-arrestin2 interaction, hence abolishing the β-arrestin2-mediated suppression of NF-κB [64]. Taken together, these findings support a function for CK2 as a crucial regulator of the NF-κB UV response.

UV irradiation was also shown to induce CK2-dependent activation of the MSK2 kinase, which is also a target of p38-MAPK in response to UV. Both CK2β and CK2α bind to MSK2 in mammalian cells after UV irradiation. However, these interactions seem to decrease after prolonged UV exposure, which might underlie temporal specificity in CK2 stress-induced signaling. CK2 phosphorylates $MSK2^{Ser324}$ *in vitro*. Phosphorylation of $MSK2^{Ser324}$ is responsive to UV irradiation and necessary for optimal UV-induced MSK2 kinase activity. Moreover, phosphorylation of $MSK2^{Ser324}$ is necessary for MSK2-mediated phosphorylation of the NF-κB subunit $p65^{Ser276}$ in response to UV light. Both these phosphorylations are required for activation of NF-κB-responsive gene expression [65]. The closely related UV-responsive MSK1 was also found to interact with both CK2α and CK2β in the cell and to be a CK2 substrate *in vitro*, but no changes were detected in MSK1-CK2 binding or CK2-dependent MSK1 activation after UV exposure [65, 66]. Direct phosphorylation of the $NF\text{-}κB^{p65}$ subunit by CK2 has been described in response to other NF-κB inducing stimuli including TNFα, Interleukin-1 (IL-1), LPS, or *H. Influenza* and *S. pneumonia* double pathogenic infection. This phosphorylation was suggested to potentiate NF-κB transcriptional activity after TNFα and IL-1 stimulation in mammalian cells [67–70]. Finally, ectopic expression of CK2α in mammalian cells leads to an increase in IKKε mRNA and protein levels, while transfection of a kinase-inactive CK2 reduces IKKε protein levels [71]. Whether this is a direct effect of CK2 in the regulation of IKKε stability is unknown. These studies have demonstrated an extensive contribution of CK2 in the regulation of NF-κB activity, functioning as both a positive and negative regulator. CK2 activity can inhibit NF-κB activation in unstressed cells as seen with pVHL-dependent CARD9 phosphorylation. Upon exposure to various stimuli, CK2 can function at multiple levels to activate NF-κB, best illustrated by CK2-mediated phosphorylation of IκBα, β-arrestin, and MSK2 in response to UV irradiation (Figure 1).

p53 stress response

The tumor suppressor transcription factor p53 plays a central role in induction of cell-cycle arrest, senescence, and apoptosis in the response to a variety of prolonged or severe stresses. In mammalian cells, p53 expression, activity, and stability are regulated by extensive PTMs and, in particular, phosphorylation of $p53^{Ser392}$ is induced in response to UV irradiation. CK2, in a complex with the chromatin factor FACT (hSPT16 and SSRP1), was identified as the kinase responsible for $p53^{Ser392}$ phosphorylation after UV exposure *in vitro*.

Association of FACT and CK2 is enhanced by UV [72]. CK2 catalytic activity towards p53 is enhanced by FACT *in vitro*, and Ser392 phosphorylation leads to an increase in p53 DNA binding and transcriptional activity in cells [72, 73]. Interestingly, since p38-MAPK activates CK2 by phosphorylation after UV induction, and since p38-MAPK inhibition abolishes $p53^{Ser392}$ phosphorylation, CK2 could act as a direct link between these two crucial signaling pathways. However, recent experiments have failed to detect any change in Ser392 phosphorylation after CK2α or p38-MAPK siRNA depletion and pharmacological inhibition, which might be due to the slow kinetics of this phosphorylation event [74]. Given these contradictory results, the physiological relevance of CK2 as the UV-induced $p53^{Ser292}$ kinase remains to be clarified. The SSRP1 subunit of FACT is also a putative UV-induced CK2 substrate that shows a decrease in DNA-binding activity when phosphorylated [75]. In maize, CK2 also phosphorylates SSRP1, which enables it to recognize damaged DNA after UV exposure [76].

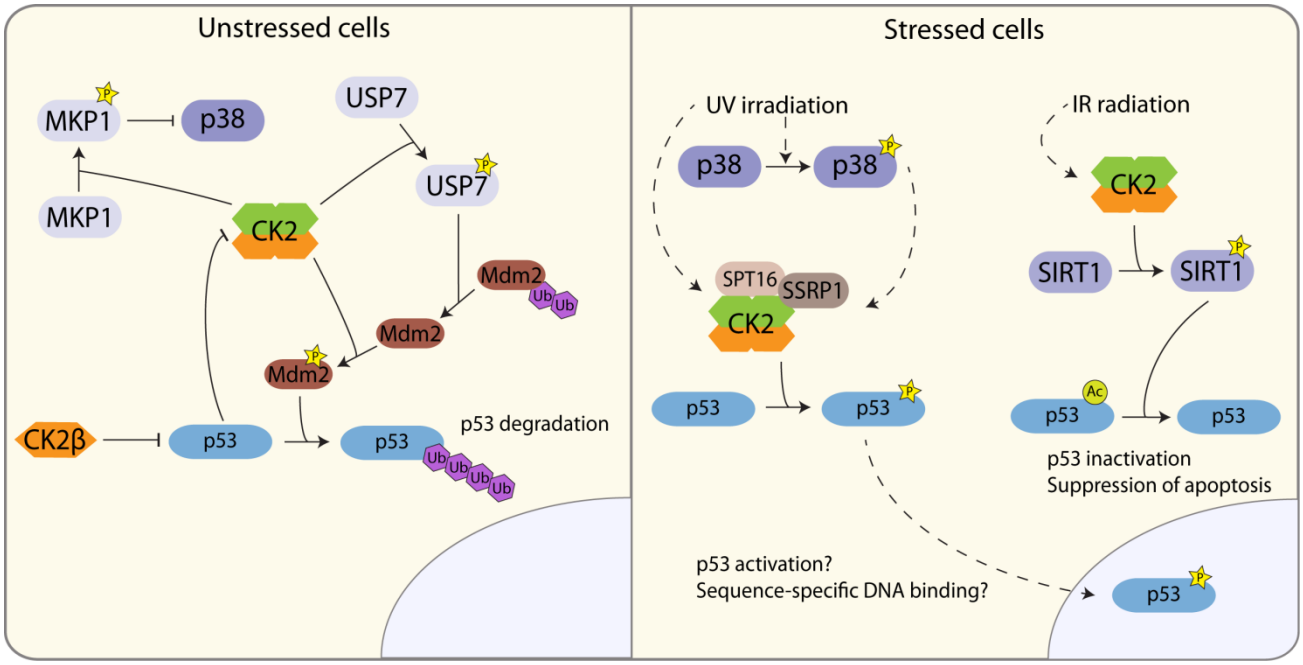

Figure 2 - Schematic representation of CK2 functions in the p53 stress response. CK2 is inhibited by p53 and promotes its degradation in the absence of stress stimuli (left panel). Upon UV irradiation CK2 phosphorylates p53 with yet unknown consequences for p53 activity, and in response to IR radiation it protects cells from p53-dependent apoptosis (right panel). Solid arrows represent direct regulatory events and broken arrows indicate molecular events that require the activity of additional factors. P – phosphorylation; Ac – acetylation; Ub – ubiquitination.

In addition to the role of CK2 in the p53 response to UV, CK2β also interacts with p53 in unstressed cells. This interaction has been suggested to reduce DNA-binding and transactivation of p53, as well as to inhibit CK2 catalytic activity [77–79]. Moreover, CK2β interacts with the checkpoint kinase 2 (Chk2) in COS-1 cells. CK2β-Chk2 interaction results in a decrease of Chk2-mediated p53 phosphorylation *in vitro* [80]. This suggests that CK2β may exert a dual inhibitory role over p53 activity, since Chk2 phosphorylation stabilizes p53 and promotes transcription of p53 target genes. Unfortunately, the physiological relevance of CK2β-Chk2 interaction was not validated, nor if its modulation by stress. Additional control of the p53 tumor suppressor

by CK2 could occur through phosphorylation-dependent stabilization of the main p53 negative regulator Mdm2. This protein is responsible for continued ubiquitination and degradation of p53 in unstressed cells, and is efficiently phosphorylated at Ser267 by CK2 *in vitro*. Expression of a phosphoresistant $Mdm2^{Ser267}$ mutant in different cell lines suggests that phosphorylation of this residue is required for optimal p53 degradation [81, 82]. Mdm2 is also targeted to degradation by the ubiquitin-proteasome system and this has been shown to depend to CK2 activity. CK2 phosphorylates the ubiquitin-specific protease 7 (USP7) *in vitro* and in cultured cells. In unstressed cells, USP7 is involved in the continued deubiquitination of Mdm2 protecting it from degradation, subsequently promoting p53 degradation. CK2-dependent constitutive phosphorylation of $USP7^{Ser18}$ is necessary for USP7 stability, efficient ubiquitination of Mdm2 and subsequent downregulation of p53 activity in unstressed cells [83].

The deacetylase SIRT1 protects cells from apoptosis through deacetylation of many important proteins including p53. Stress-induced activation of SIRT1 provides another example of CK2 regulatory functions in p53 activity. Exposure to IR induces phosphorylation of SIRT1 by CK2, which increases SIRT1 deacetylase activity, substrate-binding affinity, and suppression of apoptosis in HEK-293 cells [84]. Concordantly, siRNAs targetting CK2α and β in glioblastoma cells lead to p53-dependent cell cycle arrest and TNF-α-induced apoptosis, caused by a decrease in SIRT1 activity [85]. Interestingly, pharmacological inhibition or siRNA knockdown of CK2 in human fibroblasts was shown to induce cellular senescence. It was suggested that this senescence phenotype is caused by a decrease in SIRT1-mediated deacetylation of p53, which triggers activation of the p53-$p21^{Cip1/WAF1}$ pathway [86, 87]. Since CK2α and α' mRNA and protein levels decrease during senescence in human cells, and since CK2α and β protein levels are reduced in tissues of older rats [88–90], the CK2-SIRT1-p53 pathway could be important in organism aging.

Even though some of the above described CK2 functions need further physiological validation, they provide strong support for CK2 as a multilevel regulator of both p53 suppression in unstressed cells, and p53 activation in stress response (Figure 2). Collectively, the multilayered CK2-mediated regulation of major cell behavior determinants, such as NF-κB and p53, illustrates the complexity of its function, and further emphasizes the importance of deciphering CK2 stimuli-driven physiological dynamics.

Proteotoxic stress response

Accumulation of misfolded proteins or protein aggregates can be highly detrimental to a cell and must be attenuated to ensure cellular viability. Several molecules and physiological conditions are known to elicit a proteotoxic stress response including hypoxia, oxidative conditions, heat shock, and proteasome inhibitors. As previously mentioned, heat shock induces an increase in CK2 activity and both nucleocytoplasmic and intranuclear CK2 relocalization [40, 41]. While the regulators of heat shock-induced CK2 dynamics remain obscure, a role in the heat shock response seems to be through interaction with the heat-shock protein 90

(Hsp90) chaperone machinery. Strong evidence from yeast and mammalian cells suggests that CK2 phosphorylates and modulates the activity of Hsp90 and other co-chaperones. Importantly CK2 is necessary for chaperone function of 'kinase chaperone' Cdc37 which controls the stability and folding of most protein kinases. On the other hand, CK2 is an Hsp90-Cdc37 client kinase in yeast and mammals and depends on this complex for optimal activity and stability at least in yeast cells [91, 92]. Since the Hsp90-Cdc37 axis is necessary for the stability of numerous kinases, the positive feedback between CK2 and Hsp90-Cdc37 could have wide-ranging consequences for cellular signaling. The CK2-Hsp90 relationship is nicely reviewed by Miyata (2009) and will not be discussed further [93].

When unfolded or misfolded proteins accumulate in the endoplasmic reticulum (ER) the unfolded protein response (UPR) is activated. The mammalian UPR is mediated by three transmembrane receptors – ATF6, PERK, IRE1α – that are kept inactive in the absence of stress by inhibitory BiP/GRP78 binding. Accumulation of exposed hydrophobic residues triggers BiP dissociation from the receptors, which allows their activation. While ATF6 appears to mediate only adaptation to ER stress, PERK and IRE1α transmit both adaptive and pro-apoptotic signals. CK2 is present in the ER of unstressed cells and no changes in localization or expression can be detected upon ER stress induction [94, 95]. Nevertheless, ER stress was found to enhance CK2 kinase activity in multiple-myeloma cells. CK2 inhibition in these cells leads to a reduction in IRE1α and BiP protein levels, and an increase in PERK activation and apoptosis [96]. These results suggest involvement of CK2 in IRE1α survival signaling and protection from ER stress-induced apoptosis. Activation of IRE1α under stress induces the accumulation of an X-box protein 1 (XBP-1) splice variant that functions as a transcription factor for ER stress-related genes including BiP. Depletion of CK2α and α' by siRNA attenuates ER stress-induced XBP-1 splicing and reduces BiP expression in glial cells, providing further evidence that CK2 positively regulates IRE1α-mediated UPR [94]. The enhanced susceptibility to ER stress-induced apoptosis on CK2 depleted cells could be explained by the observation that the pro-apoptotic transcription factors ATF4 and CHOP are upregulated in these conditions [97, 98]. PERK is a known activator of ATF4 and CHOP, and its activity was enhanced upon CK2 inhibition [96]. Additionally, ATF4 binds CK2α in cells and it has been suggested that CK2-dependent phosphorylation of ATF4^{Ser215} regulates its stability [98]. Likewise, CHOP was identified as a CK2 binding partner and *in vitro* substrate. Phosphorylation of CHOP in the presumed CK2 phosphosite decreases its transcriptional activity [99]. This putative CK2-dependent potentiation of IRE1α activity and suppression of the PERK pathway suggest an important role for CK2 in UPR regulation and cell fate in conditions of ER stress.

The proteasome and its compensatory mechanism, selective macroautophagy, are involved in the degradation of misfolded and polyubiquitinated protein aggregates. CK2 was recently identified as a suppressive kinase of Nrf1, a major transcriptional regulator of proteasome-related gene expression. Direct

CK2 regulation occurs through phosphorylation of Nrf1^{Ser497} inhibiting Nrf1-dependent transcription [100]. The adaptor protein p62/SQSTM1 binds polyubiquitin chains and mediates their transport to autophagosomes. CK2 phosphorylates p62^{Ser403} *in vitro* and in Neuro2a cells, which leads to an increase in p62 turnover. Additionally, a p62^{Ser403} phosphomimicking mutation increases p62 affinity for polyubiquitin chains, formation of p62 inclusion bodies, and macroautophagic clearance of polyubiquitinated proteins upon proteasome inhibition in cells. In support of a relevant physiological function, CK2 overexpression reduces aggregation of an aggregation-prone Huntingtin mutant in a p62^{Ser403} dependent manner [101]. In contrast with CK2-dependent downregulation of stress-induced proteasome gene expression, CK2 phosphorylation of p62 appears to play a protective role in mammalian cells. The histone deacetylase HDAC6 is also involved in this process, promoting the transport and clearance of protein aggregates. There is *in vitro* evidence that CK2 is able to bind HDAC6, phosphorylates Ser458, and promotes HDAC6-dynein association. Moreover, the authors suggest that phosphorylation of HDAC6^{Ser458} increases its deacetylase activity, and promotes formation and clearance of stress-induced protein aggregates [102]. Together, these results indicate that CK2 regulates both proteasomal degradation and selective macroautophagy of aberrant proteins, and promotes a macroautophagic response in stressed cells. Concludingly, by regulating cytosolic and ER misfolded/aggregate protein clearance mechanisms, CK2 emerges as a putative coordinator of the cellular proteotoxic stress response (Figure 3).

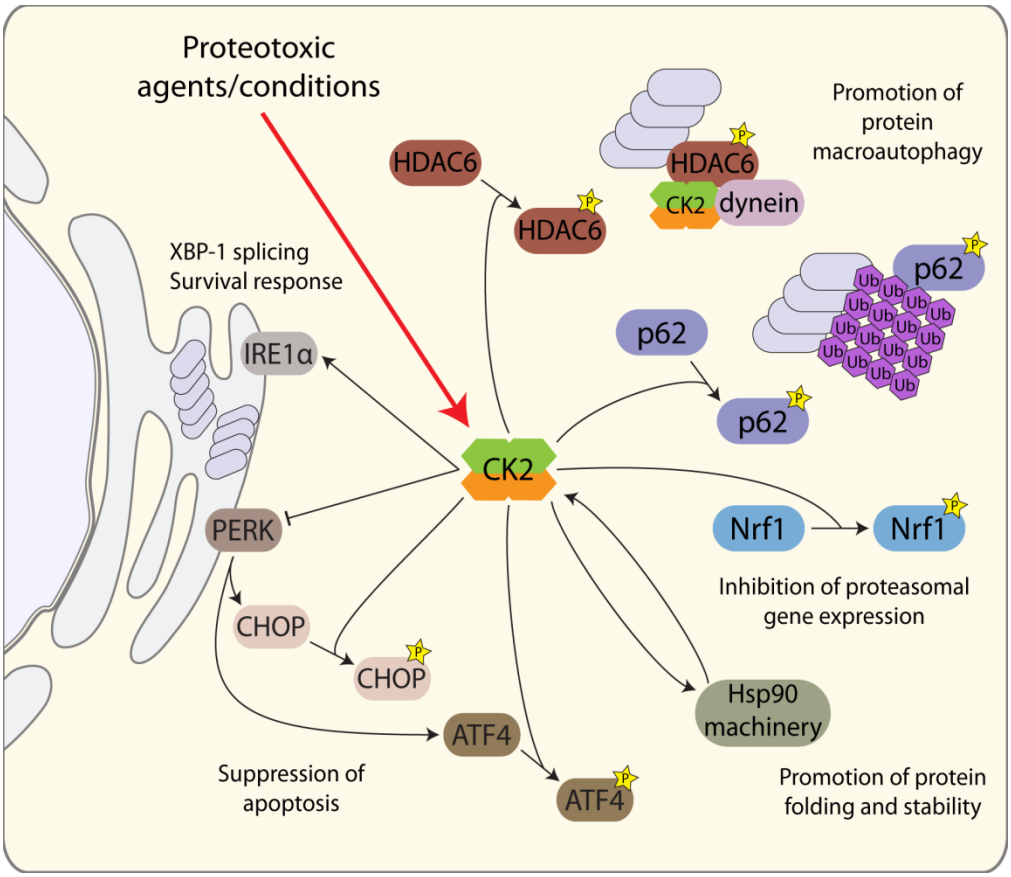

Figure 3 – Schematic representation of CK2 functions in the proteotoxic stress response. Cell exposure to proteotoxic stress-inducing chemicals or environmental conditions induces CK2 activity towards proteins involved in ER stress response, selective macroautophagy, and protein folding and stability. Solid arrows represent direct regulatory events. Red arrow indicates proteotoxic stress-dependent regulation of CK2 activity. P – phosphorylation; Ub – ubiquitination.

Genotoxic stress response

Variability generated through genomic instability is a prerequisite for evolution. Nevertheless, cells could not survive without mechanisms that mitigate most of the DNA damage and ensure fidelity in the transcriptional and generational transmission of genetic information. Cells constantly suffer DNA lesions that can arise spontaneously (e.g. deamination or depurination of DNA bases), induced by environmental conditions (e.g. UV, IR, oxidative damage), or by errors during replication. Different cellular machineries - collectively termed DNA-damage response (DDR) – are involved in detection, signaling, and repair of distinct genotoxic lesions. Additionally, the cell cycle is arrested upon DNA damage to allow for increased time for repair, and when the damage exceeds the cell's repair capabilities programmed cell death can be induced. Studies in yeast, plants, and vertebrates indicate that CK2 plays a conserved role in the regulation of many DDR pathways and genomic stability.

In budding yeast, genotoxic stress caused by DNA alkylation (methyl-methanesulfonate (MMS)-induced) or DNA double-strand breaks (DSBs; phleomycin-induced) leads to phosphorylation of Ser1 in histone H4 in the proximity of DNA damage. Proteomic studies identified CK2 as a histone H4 after MMS-induced binding partner. Recombinant CK2 can phosphorylate $H4^{Ser1}$ *in vitro*, and MMS-induced phosphorylation of this site is greatly diminished in a *cka1* null yeast strain and barely detectable in a *cka1*$^{-/-}$ *cka2*^{ts} double mutant at the restrictive temperature. It was further shown that phosphorylation at this site promotes DSB repair through non-homologous end joining (NHEJ) [103]. This suggests that histone H4 is a CK2 substrate *in vivo* and that CK2 functions in the initial steps of DDR by modifying the 'histone code' and repair mechanism preference. Other studies with budding yeast suggest that CK2 regulates adaptation to the G_2/M phase DNA damage checkpoint, a process in which the cell cycle progression is resumed even if double-strand breaks (DSBs) remain unrepaired. Accordingly, CK2β (*ckb1* or *ckb2*) null mutants faced with and irreparable DSB lead to permanent DNA-damage checkpoint arrest, which is suppressed upon DNA-damage checkpoint inhibition [30]. In Arabidopsis, inducible expression of a dominant-negative mutant of CK2α confers hypersensitivity to many genotoxins (UV, IR, MMS, and bleomycin). Moreover, CK2 inhibition leads to a decrease in homologous recombination (HR) repair and preferential use of non-conservative repair mechanisms such as NHEJ, suggesting a role in plant DSB repair and activation of HR [104]. Curiously, this CK2 mutation also enables DSBs to be repaired with higher proficiency, putatively positioning CK2 as a regulator of the delicate balance between genomic stability and instability.

The first indication of a putative role for CK2 in a mammalian genotoxic stress response came with the observation that ectopic expression of CK2β in xeroderma pigmentosum cells is able to confer partial resistance to UV irradiation [105]. Later studies showed that CK2 activity is enhanced after UV exposure, and that UV irradiation is followed by relocalization of CK2 to specific nuclear structures [40]. Dynamic

mobilization of CK2α and CK2α' to perinuclear and nuclear regions, respectively, is also observed following IR [42]. In mammalian cells, CK2α or α' siRNA knockdown leads to delayed DNA repair and persistent phosphorylation of histone H2AX (γ-H2AX), one of the earliest markers for DNA DSBs that triggers recruitment of DDR machinery [106, 107]. Additionally, knockdown of either catalytic subunit in IR-stimulated cells leads to caspase-dependent apoptotic cell death [42]. Further support for a role in DDR comes with the observation that CK2α overexpression accelerates DNA repair, and that CK2 colocalizes with γ-H2AX at DSBs after genotoxic stress [107]. These observations support a function for CK2 in mammalian DDR and genotoxic stress tolerance, which has been a target of extensive research in recent years.

Several molecular links between CK2 and DDR machineries have been reported that might explain the impaired DNA repair phenotype of CK2 knockdown. After IR or etoposide exposure, histone 3 trimethyl Lysine9 (H3K9me3)-bound heterochromatin protein 1β (HP1-β) swiftly mobilizes from chromatin and facilitates H2AX phosphorylation. Studies *in vitro* and in cell cultures show that CK2 phosphorylates HP1-β^{Thr51}, which modulates HP1-β affinity for H3K9me3 after DNA damage. Pharmacological inhibition or overexpression of a dominant-negative CK2α in damaged cells abolishes Thr51 phosphorylation, and mimics a chromatin-bound HP1-β mutant, leading to diminished γ-H2AX signal and inefficient DNA repair. These results suggest that CK2 is necessary for displacing HP1-β from the chromatin, putatively allowing access of the repair machinery to DNA lesions [106]. Together with CK2-mediated $H4^{Ser1}$ phosphorylation in yeast, these studies implicate CK2 in the regulation of the earliest known cellular events upon genotoxic stress. The transcriptional gene silencing functions of HP1 are also modulated by CK2 activity in *Drosophila* and fission yeast, and will be discussed in the following chapters [108, 109].

Subsequent steps in DSB repair include ATM-dependent recruitment of the Mre11-Rad50-Nbs1 (MRN) complex to damaged chromatin marked by γ-H2AX. The Forkhead (FHA) BRCA1 C-terminus (BRCT) domain-containing protein mediator of DNA damage checkpoint protein 1 (MDC1) binds to γ-H2AX and is necessary for MRN recruitment to DNA lesion sites. MDC1 is constitutively phosphorylated at several acidic motifs *in vivo*, and the same sites are efficiently phosphorylated by CK2 *in vitro* [110–113]. Three independent groups have shown that constitutive phosphorylation of MDC1 is necessary for MDC1-Nbs1 interaction, hence MRN recruitment to damaged DNA foci. In support of a CK2 function in MDC1 signaling, double siRNA knockdown of CK2α and CK2α' abolishes the MDC1-Nbs1 interaction in the nucleus, MRN accumulation in damaged chromatin, and intra-S-phase checkpoint activation [111, 112, 114]. CK2-dependent phosphorylation of MDC1 was also reported to be essential for MDC1 binding to another FHA protein Aprataxin, with consequences for single-strand break (SSB) repair [115].

In addition to this role in the ATM-dependent arm of DNA repair, CK2 regulates the two other main pathways for repair of DSBs, HR and NHEJ, and also base excision repair (BER) of SSBs. Repair through HR is

restricted to S and G2 phases of the cell cycle since it requires a sister chromatid for recombination. The recombinase Rad51 is necessary for catalyzing homologous pairing and strand transfer during HR repair. Cell cycle-dependent activity of Polo-like kinase 1 (Plk1) drives phosphorylation of Rad51^{Ser14} and is a prerequisite for subsequent Rad51^{Thr13} phosphorylation by CK2. This hierarchical phosphorylation occurs in cells after IR, and is responsible for Rad51 recruitment to damaged DNA via Nbs1 binding and promotion of HR repair [116]. Since Plk1 activity is restricted to specific cell cycle phases, hierarchical phosphorylation primed by Plk1 reveals a mechanism for CK2 temporal regulation.

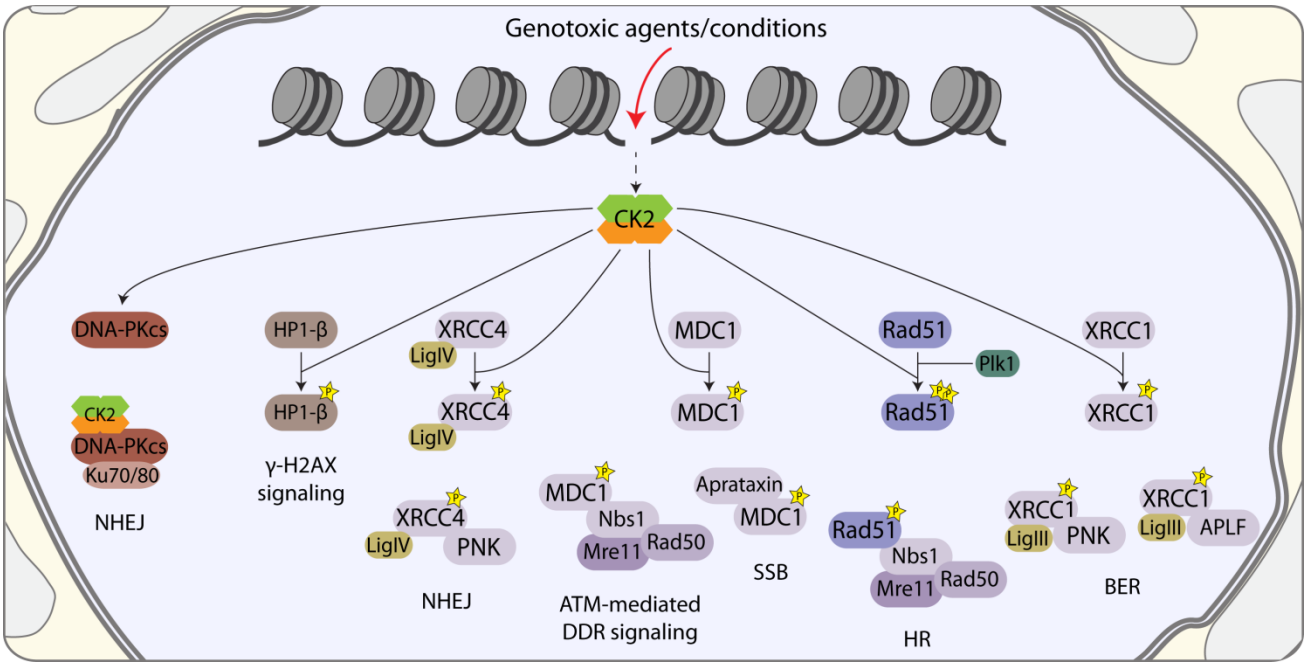

Figure 4 - Schematic representation of CK2 functions in the genotoxic stress response. Cell exposure to genotoxic stress-inducing molecules or environmental conditions induces CK2 regulation of diverse DNA repair mechanisms. FHA domain proteins whose interactions are regulated by CK2-mediated phosphorylation are depicted in light blue boxes. Solid arrows represent direct regulatory events and broken arrows indicate indirect events. Red arrow represents lesions in the DNA. P – phosphorylation.

The non-conservative repair mechanism NHEJ relies on the Ku70/80 heterodimer that recognizes DNA lesions and recruits other components such as the DNA-dependent protein kinase catalytic subunit (DNA-PKcs). Autophosphorylation of DNA-PKcs occurs shortly after its recruitment and leads to its release from DSBs, which is postulated to allow higher accessibility of other repair molecules to damaged DNA. In DNA-PKcs-proficient human cells, siRNA against CK2α induces apoptosis, decreases DNA-PKcs autophosphorylation, delays DNA repair, and leads to persistence of γ-H2AX signal. CK2 associates with DNA-PKcs in cells, and this interaction is enhanced by genotoxicity. Moreover, CK2 inhibition greatly reduces DNA-PKcs-Ku70/80 interaction [107]. From these results, CK2 seems to be required for proper DNA-PKcs signaling in response to genotoxic stress. NHEJ repair also depends on the putative CK2-dependent constitutive phosphorylation of the adaptor XRCC4, another FHA-domain protein. XRCC4 is usually in a complex with DNA ligase IV and in genotoxic conditions recruits the polynucleotide kinase (PNK) to damaged DNA. Formation of the DNA ligase IV-XRCC4-PNK complex is important for the repair and rejoining of damaged DNA termini.

Phosphorylation of XRCC4 at the CK2 phosphosite enables XRCC4 to interact and recruit PNK to sites of DNA damage [117]. CK2 functions in a similar fashion in base excision repair (BER) and SSB repair through constitutive phosphorylation of another adaptor FHA-containing protein, XRCC1. Phosphorylation of XRCC1 is required for efficient DNA repair, as well as for XRCC1-DNA Ligase III complex stability, XRCC1-PNK and XRCC1-APLF FHA-mediated interactions, and XRCC1 release from DNA [118–121].

In conclusion, CK2 activity has been implicated in most phases of DNA damage signaling including early chromatin and histone modifications, potentiation of repair mechanisms, and cell fate decisions induced by severe damage (Figure 4). Collectively, these studies argue for a conserved CK2 function in the promotion and coordination of DNA damage-induced cellular responses. Upon CK2 depletion, the DNA damage-induced γ-H2AX signal was weaker but more persistent, and DNA repair was delayed, suggesting that CK2 regulates the timing and duration of the DDR response. Notably, CK2 controls phosphorylation-dependent FHA domain-mediated interactions with DDR scaffolding proteins, playing a key role in the recruitment of HR, NHEJ, ATM-dependent, and BER repair machineries. Analysis of CK2 subunit dynamics and interactions in cells exposed to genotoxic stress should help elucidate how CK2 contributes to the activation of specific DDR pathways. Moreover, since phosphopeptide-binding FHA domains are widespread among regulatory proteins, it is tentative to speculate that CK2 might have a broader regulatory impact on FHA-dependent interactions.

Cell polarity

Establishment of polarity is an essential and ubiquitous process that generates variability and complexity in living systems. Cell polarity requires the asymmetric distribution of the cell's molecular contents, which generates subcellular domains with different molecular compositions. Polarity establishment is required in multiple developmental processes such as regeneration of undifferentiated cells through asymmetric cell division, tissue and body patterning, or subcellular functional specialization in differentiated cells. Polarity can be an intrinsic property of a cell, generated by self-organizing molecular networks, or induced by external stimuli through activation of signal transduction pathways. Seemingly in contrast with its ubiquitous intracellular distribution, CK2 emerged as an important regulator of eukaryotic cell polarity.

A contribution of CK2 to polarity generation was first described in budding and fission yeast cells. After septation, fission yeast rod-shaped daughter cells grow in length by extension of 'old' ends in a polarized fashion. Snell and Nurse (1994) identified a mutation, later associated with the *ck1a* gene, which gave rise to spherical cells defective in cell elongation. This phenotype is detectable only after cytokinesis indicating that CK2 is not necessary for establishment of polarity but rather for reinitiation of polarized growth after septation [25]. In *S. cerevisiae*, bud emergence depends on the polarized assembly of a cortical actin ring

followed by targeting of secretory vesicles to the presumptive bud site. Whereas a *cka1*^{ts} strain displays a spherical morphology at the restrictive temperature, *cka2*^{ts} cells hyperpolarize after temperature shift. Even though the reason for these dissimilar phenotypes is not clear, both mutants were defective in polarization of the actin cytoskeleton, suggesting a role for CK2 in polarity establishment [28]. Most of the progress in elucidating CK2 molecular involvement in polarity has been made using mammalian cell cultures. In this section, the contribution of CK2 to epithelial and neuronal cell polarity will be discussed.

Epithelial cell polarity

Epithelial cells organize into tight sheets that surround the inner and outer cavities of metazoans, functioning as a barrier and as a place for molecular exchange between the organism and the environment. An actin-rich apical domain and a basolateral domain characterize a polarized epithelial cell. These domains have distinct morphologies and functions and are separated by a subapical belt of junction complexes that form cell-cell and cell-matrix contacts. Current models on epithelial polarity suggest a preponderant role for junction assembly and stabilization in symmetry-breaking, which is followed by distribution of cross-regulatory and self-perpetuating polarity-determinant protein complexes. In mammalian epithelial cells, a fraction of CK2 localizes to the plasma membrane through an interaction with the pleckstrin homology domain of CK2-interacting protein 1 (CKIP1) [122, 123]. Pharmacological inhibition of CK2 or CK2β siRNA was shown to induce loss of cell adhesion and cell-cell contacts eventually inducing a mesenchymal-like morphology [123–125].

Cell-matrix and cell-cell contacts are necessary for polarization and depend on the dynamic assembly of adhesion and junction protein complexes. Similarly, depolarization or acquisition of a mesenchymal morphology requires disassembly of junction complexes. Occludin localizes to tight junctions, establishes cell-cell contacts, and is important in determining the permeability characteristics of epithelial sheets. Human, mice, and *Xenopus* CK2 proteins are able to phosphorylate occludin at three residues *in vitro*, and a direct interaction was detected by co-immunoprecipitation in human cells [126, 127]. Although the physiological relevance of CK2 as an occludin kinase is unclear, a phosphomimetic occludin mutant at the three putative CK2 sites shows delayed reassembly dynamics and enhanced barrier properties [126]. Interestingly, as an ectokinase CK2 might play another role in promoting cell-adhesion by phosphorylating components of the ECM. Vitronectin and laminin-1 are ECM proteins and CK2 substrates *in vitro*. Phosphorylation of vitronectin at the presumed CK2 phosphosites was suggested to enhance adhesion properties [128]. The physiological relevance of CK2 activity in the regulation of adhesion molecules is still not understood. However, since CK2 export as an ectokinase depends on CK2β [129], and since CK2β knockdown causes loss of epithelial adhesion, CK2 might indeed be a physiological regulator of junction and adhesion molecules. A subsequent step required for epithelial polarity is LKB1/Par4-mediated recruitment/assembly of apical determinant complexes. Knockdown of CK2β was sufficient to inhibit

polarization after STRAD induction of LKB1, revealing CK2 as an essential player in the establishment of epithelial polarity [123, 125].

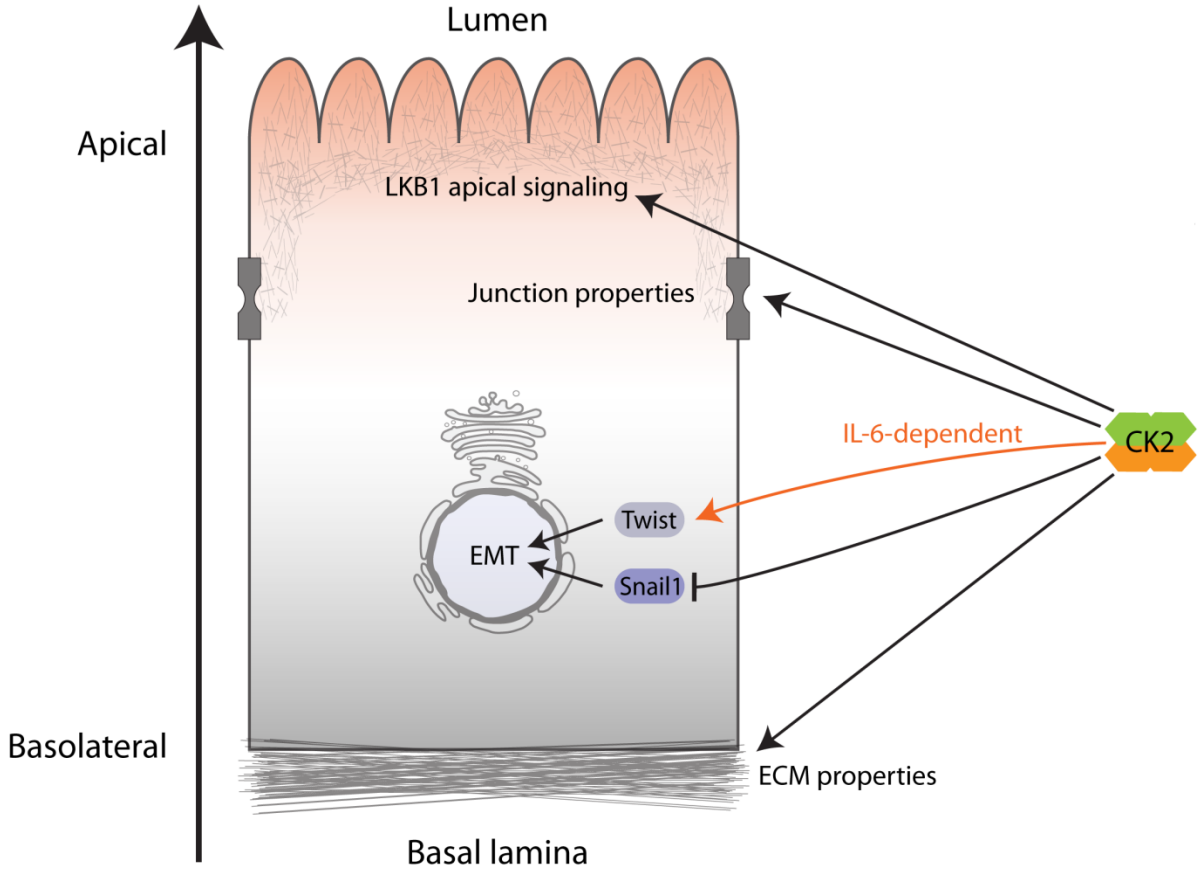

Figure 5 – Functions of CK2 in the regulation of epithelial cell morphology and plasticity.

The mesenchymal phenotype of CK2β reduction in epithelial cells is in accordance with the positive correlation between CK2α overexpression and metastatic risk in breast carcinoma [130]. Recently, it was shown that the mesenchymal phenotype of CK2β requires the transcription factor Snail1. Snail1 - together with the transcription factor Twist - acts as a convergence point of many pathways that promote an epithelial-to-mesenchymal transition (EMT). Interaction between CK2 and Snail1 occurs through CK2β. Promoted by CK2β, CK2 phosphorylates Snail1, thereby priming a subsequent phosphorylation by GSK3β. This CK2-dependent double phosphorylation inactivates Snail1 and promotes maintenance of epithelial morphology [124]. In contrast to its signaling in unstimulated cells, CK2 was reported to phosphorylate and stabilize Twist upon IL-6 cytokine stimulation, thereby inducing EMT [131]. Although interesting, these results are not conclusive as of yet since a non-specific CK2 inhibitor was used in cell culture assays. Reduction of CK2β resulting in an unbalance of CK2 subunits was necessary to drive EMT through Snail1, but the function of CK2β in Twist activity was not accessed. A noteworthy fact is that Snail1 is an NF-κB downstream target, which would suggest that CK2β might exert a negative effect on NF-κB activity in

unstressed cells, as seen for p53. Taken together, the described functions for CK2 in the regulation of both polarization and EMT implicates this enzyme in the control of epithelial plasticity (Figure 5).

Neuronal polarity

Neurons polarize to guarantee a unidirectional flow of information from the somatodendral to the axonal compartment, which is crucial for proper functioning of neural networks. CK2 contribution to neuronal development and polarity was first noticed when CK2-depleted neuroblastoma cells failed to undergo neuritogenesis. This depletion was accompanied by dephosphorylation of the microtubule-associated protein MAP1B and its release from microtubules [132]. MAP1B is a CK2 substrate *in vitro*, however it is not clear whether CK2 can control MAP1B-dependent microtubule dynamics *in vivo*. CK2 itself is a *bona fide* microtubule-associated protein (MAP) that promotes tubulin polymerization and microtule stability [133]. Just as in epithelial polarity, cellular adhesion is very important for the development of functional neural networks. L1CAM is a transmembrane adhesion molecule involved in axon elongation, guidance, and neuronal migration. CK2 phosphorylates L1CAM *in vitro* at CK2 consensus sites detected *in vivo* [134]. Subsequent studies showed that phosphorylation of L1CAM at Ser1181, one of the putative CK2 sites, greatly influences its activity. Phosphorylation of $L1CAM^{Ser1181}$ promotes endocytic trafficking of L1CAM, axon elongation, and binding of L1CAM to the signaling scaffold protein 14-3-3ζ. Curiously, presence of 14-3-3ζ enhanced CK2 activity towards L1CAM suggesting that 14-3-3ζ might facilitate CK2 accessibility to L1CAM or functions as a regulator of CK2 target specificity [135, 136]. Since L1CAM endocytic trafficking has been described as a critical step for axon elongation [137], the CK2-14-3-3ζ axis might play a decisive part in formation of neural networks.

Among the earliest events in neuronal symmetry-breaking is the formation of the axon initial segment (AIS). This region of the axon is structurally different from other cellular regions, and is characterized by unusual microtubule properties (high acetylation, low tyrosination) and a cluster of ion channels that generate and amplify action potentials. Accumulation of ion channels such as $Na_v1.2$ and maintenance of the AIS are dependent on AnkyrinG, a cytoskeletal linker, and on the IκBα-IKK pathway. In cultured hippocampal neurons, knockdown of CK2 by siRNA increases cellular microtubule acetylation, inhibits axonal elongation, and impairs the distal axonal distribution of the CK2 substrate kinesin KIF5C [138–141]. Moreover, CK2 knockdown decreased its own accumulation in the AIS, as well as accumulation of AnkyrinG, IκBα, and voltage-gated sodium channels [139, 140]. Concordantly, imaging of CK2 subcellular localization in developing neurons reveals high enrichment of CK2 protein levels in the AIS and nodes of Ranvier, despite its ubiquitous presence [139, 140]. Depletion of AnkyrinG or suppression of IκBα activity impairs CK2 targeting to the AIS, suggesting that AIS accumulation of AnkyrinG, IκBα, and CK2 are mutually dependent [140]. An important function described for CK2 in AIS establishment is the direct regulation of ion channel clustering. CK2 phosphorylates $Na_v1.2$ channels within their ankyrin-binding motif *in vitro*, strongly increasing their

binding affinity towards AnkyrinG [139]. In agreement, disruption of putative CK2 phosphosites within the $Na_v1.2$ and the $Na_v1.6$ ankyrin-binding motif increases sodium channel axonal diffusion and impairs AIS channel clustering [142, 143]. Overall, these results argue for an important function of CK2 in AIS formation, neuronal polarity, and neuritogenesis (Figure 6).

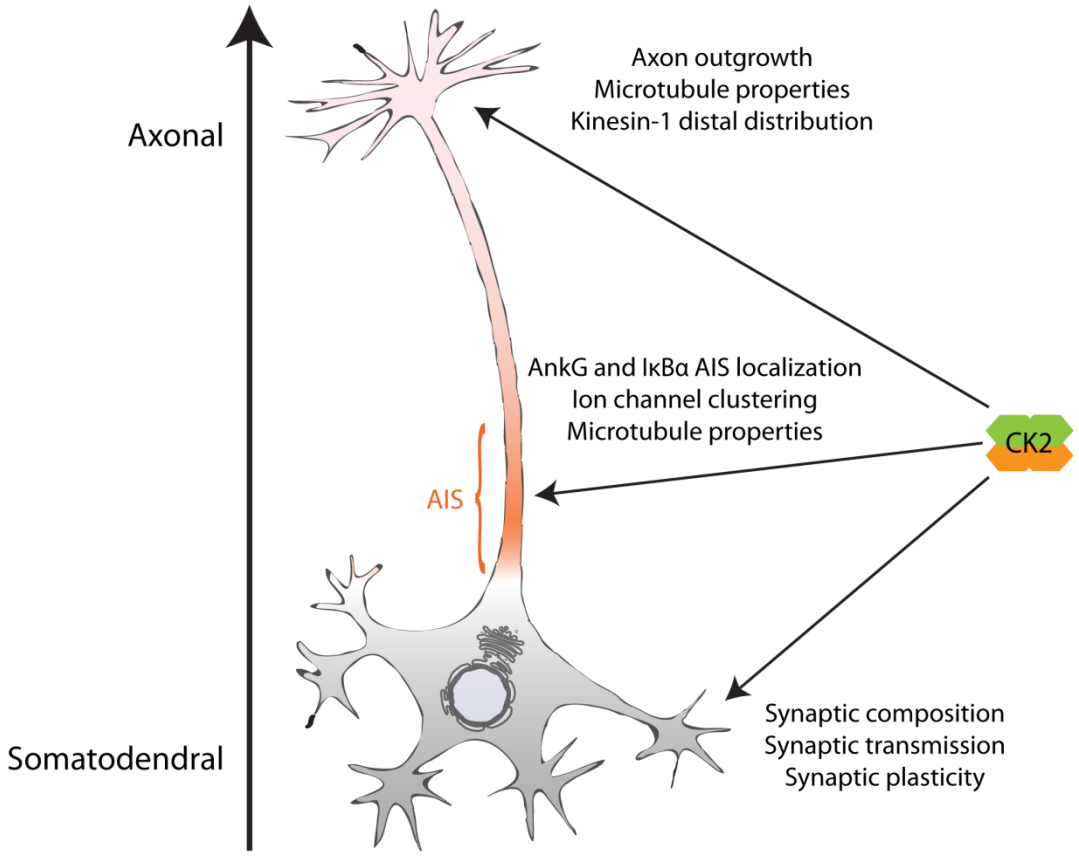

Figure 6 - Functions of CK2 in the regulation of neuritogenesis and neuronal function.

Curiously, inhibition of the above-mentioned CK2 target HDAC6 in hippocampal neurons also impairs AnkyrinG and sodium channels AIS accumulation as well as distal KIF5C axonal distribution and axonal elongation. Upon HDAC6 inhibition the overall levels of microtubule acetylation were increased, similarly to what occurs when CK2 activity is inhibited. However, in hippocampal neurons the tubulin deacetylase activity of HDAC6 is restricted to the distal region of the axon, which putatively allows the formation of acetylated microtubule domains such as the AIS [144]. Since CK2 and HDAC6 have partially complementary axonal expression patterns, and since CK2-dependent $HDAC6^{Ser458}$ phosphorylation was suggested to increase deacetylase activity, another mechanism should be regulating HDAC6 activity or Ser458 availability in the AIS. It is currently unknown if $HDAC6^{Ser458}$ phosphorylation is restricted to stress response, and if there is any physiological relationship between these two proteins in AIS development, as suggested by the similarity of knockdown phenotypes.

The emerging picture is that CK2 activity is a determinant for proper neuronal morphogenesis and function. Indeed, CK2 has been implicated in the regulation of synaptic composition, transmission, and plasticity, impacting processes such as spatial memory formation and long-term potentiation [145–150]. Although these topics will not be further discussed, they highlight the importance of CK2 in the nervous system, and in the coordination of finely tuned and dynamic processes.

Survival, death, and cell cycle progression

As previously described, CK2 is a key regulator of the survival and death decisions imposed on cells by stressing conditions. It turns out that CK2 plays a much broader role in the integration of signals that mediate growth, proliferation, and death in developing cells. This is not surprising given the mechanistic similarity and necessary overlap between stress and developmental signaling. Mammalian cell growth and proliferation depends on nutrient availability and is stimulated by growth factors. However, in the absence of nutrients or growth factors, cell development must be quickly arrested to avoid unnecessary waste of metabolites and to increase chances of long-term survival. Just as for stressing conditions, prolonged lack of growth stimuli can trigger activation of programmed cell death pathways. Expression levels and activity of CK2 seem to be positively correlated with favorable conditions for cell growth, as evidenced by the higher expression in proliferating tissues and cancer cells when compared to resting cells [151]. Stimulation of cells with mitogens or growth factors results in elevated CK2 activity and partial relocalization to the nucleus [152–157]. Knockdown or pharmacological inhibition of CK2 blocks cell cycle progression and inhibits cell growth induced by growth stimuli [158–160]. Withdrawal of growth stimuli leads loss of CK2 from the nuclear fraction and induction of apoptosis [161, 162]. A regulatory function for CK2 in the in the insulin response and carbohydrate metabolism was recently described, suggesting that CK2 can directly coordinate growth stimuli reception with modulation of cellular metabolism (reviewed in [163]). CK2 involvement in cell growth, proliferation, and death is probably the best described facet of this pleiotropic signaling molecule, given its relevance for tumor biology. In this chapter, the cellular activity of CK2 in mitogenic signaling, programmed cell death, and cell cycle progression will be discussed.

Cell growth and survival

The ERK MAPK pathway is responsible for the signal transduction of many mitogens such as growth factors and hormones that promote cell survival. Mitogens captured by cell surface receptors trigger Ras GTPase activity that promotes activation of the kinase Raf. A phosphorylation cascade is ensued, with Raf activating the kinase MEK which subsequently activates ERK. This is followed by ERK nuclear translocation where it regulates transcription factors that promote cellular changes, such as cell-cycle progression. Epidermal growth factor (EGF) was one of the first known inducers of CK2 catalytic activity [152, 164]. The receptor EGFR and the ERK pathway mediate transduction of EGF signaling. Recently, it was reported that ERK2 binds

and phosphorylates CK2α at Thr360 and Ser362 *in vitro* and tissue culture, enhancing CK2 catalytic activity [164].

Downstream of EGFR, signaling is coordinated by the scaffold protein kinase suppressor of Ras 1 (KSR1) that translocates from the cytosol to the plasma membrane upon Ras activation. This protein functions as assembly point for ERK pathway kinases, binding Raf, MEK, and ERK and facilitating signal transduction. CK2 and KSR1 are constitutively bound in mammalian cells, independently of growth factor treatment, and this interaction is necessary for proper activation of MEK and ERK. The interaction between KSR1 and CK2 is needed for CK2-dependent phosphorylation of B-Raf^{Ser446} and C-Raf^{Ser338}, and subsequent activation of the ERK pathway in response to EGF [165]. Similarly, *Drosophila* KSR and CK2 homologs interact *in vivo*, leading to CK2-mediated activating phosphorylation of Raf. Activation of Raf by CK2 in *Drosophila* is modulated by polyamines, revealing a direct link between polyamine levels and CK2-dependent activation of ERK mitogenic signaling [166]. Polyamines are essential for cellular growth and their levels usually reflect nutrient abundance and favorable growth environments. Polyamines directly stimulate CK2 activity *in vitro* and in mammalian cells, and increase CK2 uptake into purified nuclei [154, 167]. In support, overexpression of ODC, the rate-limiting enzyme in polyamine biosynthesis, leads to higher CK2 activity in transgenic mice, which is abolished upon ODC inhibition [168, 169].

The mammalian A-Raf is a CK2β binding partner. CK2β was found to increase A-Raf activity in cell culture and coexpression of CK2α abolishes this increase [170]. This is in contrast to the CK2-mediated potentiation of B- and C-Raf and suggests that CK2β can contribute to A-Raf regulation independently of the CK2 holoenzyme. The closely related kinase Mos can also activate MEK after mitogenic stimulation and bind to CK2β in a physiological context. In contrast to Raf-CK2β interaction, Mos activation of MEK during Xenopus oocyte maturation is inhibited by CK2β, and this inhibition is alleviated by CK2α [171]. These results underscore the importance of considering the effects of individual subunits when investigating CK2 cellular functions.

CK2 interacts with ERK2 in cells and mitogens stimulate this interaction. CK2 was suggested as the main cellular kinase of two phosphosites within the ERK2 nuclear translocation signal (NLS), which are necessary for ERK nuclear import. The putative CK2 phosphorylation of ERK is necessary for efficient ERK2 binding to the nuclear importer Imp7. Furthermore, CK2α knockdown or expression of a phosphoresistant ERK inhibit ERK2-mediated phosphorylation of nuclear targets, an effect not observed in ERK cytoplasmic targets [172]. These results demonstrate a specific role for CK2 in regulating ERK2 nuclear functions. Inhibitory functions of the ERK phosphatases MKP3 and PP2A have been described as targets for CK2 regulation. MKP3 binds to CK2α in cells and is phosphorylated by CK2 *in vitro*. MKP3 phosphatase activity towards ERK2 is reduced when coexpressed with CK2α in mammalian cells after EGF stimulation, in a CK2 kinase-independent manner [173]. PP2A binds CK2α in cells, but not when CK2β is present or when platelet-derived growth factor (PDGF)

is added to the culture media. CK2α phosphorylates PP2A, and stimulates its activity towards MEK in a dose-dependent manner, thereby inhibiting the MAPK cascade [174]. Since growth factor stimulation and CK2β abolished CK2α-PP2A interaction, CK2α-mediated stimulation of PP2A phosphatase activity might be a mechanism to prevent MAPK activation in unfavorable mitogenic conditions.

The EGFR-ERK pathway is commonly hyperactivated in cancers and associated with promotion of EMT and metastasis in tumor cells. Proteins of the p21-activated kinases (PAKs) family are known effectors of small GTPase signaling and regulate various cellular processes including cell cycle progression and actin cytoskeleton dynamics. PAKs can also be activated by GTPase-independent stimuli such as EGF, sphingolipids, PIP_2, or the PDK1 kinase. Contact with its activators triggers a conformational change in PAK1 that converts an inactive dimer into an active monomer. It was demonstrated that this is not sufficient for PAK1 activation following GTPase-dependent and GTPase-independent stimuli. In fact, CK2 phosphorylation of $PAK1^{Ser223}$ is necessary for activating monomeric PAK1 after both kinds of stimuli, including EGF stimulation. PAK1-mediated tumor anchorage-independent growth is induced by expression of wild-type PAK1 but not of a phosphoresistant $PAK1^{Ser223}$ mutant, in both cell culture and in mice xenographs [175]. These results strongly suggest that CK2 is a major physiological regulator of PAK1 activity, including EGF-induced actin cytoskeleton dynamics in cell adhesion and motility.

The consequences of ERK phosphorylation of $CK2\alpha^{Thr360/Ser362}$ provide further support for CK2 as a coordinator of cell survival. It was shown that EGF/EGFR activation induces ERK2-CK2α interaction and enhances CK2 catalytic activity via ERK2-depedent phosphorylation of $CK2\alpha^{Thr360/Ser362}$. The cell-adhesion molecule α-catenin is a preferential target for EGF-induced CK2 activity. CK2-dependent phosphorylation of $\alpha\text{-catenin}^{Ser641}$ results in the disruption of the α-catenin-β-catenin complex. Importantly, disruption of this complex through the EGF-induced ERK2-CK2 pathway was shown to promote β-catenin transactivation and tumor cell migration [164]. Together with the regulation of Snail1 and Twist, this CK2 activity highlights its contribution to the control of epithelial plasticity. β-catenin is part of the cadherin-catenin cell adhesion complex, and is a crucial component of the Wnt/β-catenin signaling pathway. While Wnt/β-catenin signaling mediates fundamental developmental processes such as cell proliferation, differentiation, and patterning, aberrant Wnt/β-catenin activation often leads to transformation. Research in different models suggests that CK2 is widely involved in the regulation of this multifunctional pro-survival pathway.

In *Xenopus*, CK2 is necessary for the Wnt/β-catenin-mediated dorsal axis formation. Injection of CK2α/β mRNA in ventralized blastomeres is sufficient to induce a complete dorsal axis, whereas expression of a kinase-inactive CK2α blocks endogenous Wnt/β-catenin-dependent dorsal axis formation [176]. Recently it was shown that an additional component of the cadherin-catenin complex is an endogenous suppressor of CK2 in Wnt/β-catenin signaling. This is mediated by the paraxial protocadherin (PAPC) which binds CK2β and

suppresses CK2-induced Wnt/β-catenin dorsalization of *Xenopus* oocytes [177]. Activation of the Wnt/β-catenin requires the binding of small extracellular ligands of the Wnt family to Frizzled (Fz) and LRP receptors and activation of the scaffold Dishevelled (Dsh). Activation of Dsh promotes disassembly of the β-catenin destruction complex - which includes GSK3β, APC, Axin, PP2A, and CK1α - that targets β-catenin for proteasomal degradation and prevents its cytoplasmic accumulation. Consequentially, following Wnt stimulation β-catenin, is stabilized and translocates to the nucleus, where it serves as a coactivator for the TCF/LEF family of transcription factors and promotes expression of Wnt target genes.

A strong indication that CK2 is a physiological regulator of Dsh came from studies in *Drosophila*. CK2 phosphorylates Dsh *in vivo* and this is enhanced by Wnt stimulation or Fz overexpression [178]. Similarly, CK2 phosphorylates mammalian Dsh-2/3 *in vitro*, and stimulation of mammalian cells with Wnt3 leads to a transient increase in CK2 activity [179, 180]. Additionally, CK2 was reported to phosphorylate β-catenin, thereby increasing both its stability and transcriptional activity [180, 181]. CK2 phosphorylates the transcription factor LEF-1 at two serines within the β-catenin interaction domain. Phosphorylation of LEF-$1^{Ser42/Ser61}$ decreases its affinity for the TLE1/Groucho corepressor, increases LEF-1 affinity for β-catenin, and stimulates LEF-1-β-catenin transcriptional activity [182]. This CK2-dependent stimulation of LEF-1-β-catenin also leads to upregulation of the apoptotic inhibitor survivin [183]. Survivin promotes proliferation and inhibits TRAIL-mediated apoptosis. Both of these activities are compromised by a phosphoresistant mutation of survivin^{Thr48} residue. This residue is putatively phosphorylated by CK2, suggesting that CK2 might be a relevant regulator of survivin activity [184]. The tumor-suppressor APC is part of the β-catenin destruction complex and negatively regulates many survival pathways. APC and CK2 physical association causes a potent inhibition of CK2 catalytic activity in cultured cells. Interestingly, the truncated mutant form of APC found in most cancers did not have an inhibitory effect on CK2 [185]. These results suggest that tumor-suppressor activity of APC might be in part through inhibition of CK2 pro-survival signaling.

The endocytic route followed by activated Wnt receptors can determine whether Wnt/β-catenin pathway is activated (caveolin-mediated endocytosis), or inhibited (clathrin-mediated endocytosis). Upon Wnt3 stimulation, CK2 phosphorylates the receptor LRP6, which regulates its association with the endocytic adaptor Disabled-2 (Dab-2). Dab-2 promotes inhibitory LRP6 internalization via clathrin-mediated endocytosis. CK2-mediated phosphorylation of LRP6 is necessary for Dab-2-mediated suppression of Wnt/β-catenin signaling, but does not interfere with 'activating' caveolin-mediated internalization. This suggests that CK2 can specifically suppress β-catenin signaling through promotion of Dab-2-LRP6 interaction [186]. Collectively, these results implicate CK2 as an integral component of the Wnt/β-catenin pathway, mostly potentiating β-catenin signaling. Although, as is often the case with this enzyme, physiological context and subunit dynamics may also underlie antagonistic functions.

The conserved PI(3)K/mTORC/Akt axis regulates metabolism, protein synthesis, and stress response, being one of the major pathways ensuring eukaryotic cell growth and survival. The kinase mTORC exists in two distinct cellular complexes. Whereas mTORC1 mainly promotes growth, mTORC2 restrains growth to ensure cell survival. mTORC2 survival function is mediated by activation of Akt signaling, and mTORC1 is known to negatively regulate Akt through inhibition of its upstream activator, the phosphatidylinositide 3-kinase PI(3)K. Recently, progress has been made in elucidating the molecular mechanism behind activation of mTORC2 following growth stimuli withdrawal. The Tel2 and Tti1 proteins associate with all PI(3)K-related kinases (PIKK) - ATM, ATR, mTOR, DNA-PKcs, SMG1, and TRRAP - and regulate their cellular stability. They are integral components of both mTORC1 and MTORC2 and necessary for assembly of mTORC complexes. After growth factor deprivation, CK2 undergoes nuclear-cytoplasmic translocation, and associates specifically with mTORC1 to phosphorylate mTORC1-associated Tel2 and Tti1. Phosphorylation of Tel2^{Ser485} and Tti1^{Ser828} by CK2 primes for SCFF^{bxo9}-mediated degradation of Tel2/Tti1 via the ubiquitin-proteasome system. Loss of Tel2/Tti1 inactivates mTORC1, which suppresses its inhibition of PI(3)K/Akt pathway and promotes mTORC2 signaling [187]. These results indicate that CK2 relocalization and activity, regulated by yet unknown mechanisms, coordinate the survival response to growth stimuli withdrawal and promote PI(3)K/mTORC2/Akt signaling. Tel2 is also a target of CK2 growth promoting activity in unstressed cells. Constitutive phosphorylation of Tel2$^{Ser487/Ser491}$ by CK2 creates a recognition motif for the PIH1D1 subunit of the R2TP/prefoldin-like complex. The interaction between Tel2 and the R2TP complex is necessary for stability of mTOR and the related kinase SMG1, which regulates nonsense-mediated mRNA decay [188]. The CK2-dependent constitutive phosphorylation of Tel2 and subsequent mTOR stability suggests an important function for CK2 in the maintenance of metabolic signaling pathways and cellular permissivity to growth stimuli induction.

Strong evidence suggests that phosphorylation of Ser129 of Akt in a physiological context depends on CK2 activity [189–191]. Akt^{Ser129} phosphorylation induces Akt kinase activity towards GSK3β and enhances Akt-Hsp90 interaction, putatively stabilizing Akt in its active form [189, 190]. Furthermore, CK2-dependent AKT^{Ser129} phosphorylation induces β-catenin nuclear accumulation and transcriptional activity, as well as survivin expression in human cells [191]. Direct association with CK2β and kinase-dead CK2α drastically enhance Akt activity, indicating that CK2 can promote Akt activation independently of the kinase activity [192]. These results suggest that CK2 is a direct inducer of Akt activity and stability, and sustains an Akt-mediated survival response. Zinc signaling is a recently characterized signaling pathway that is responsive to extracellular stimuli that promote activation of growth and survival signaling pathways. Stimulation of human cell lines with zinc or EGF quickly induces an association between CK2 and the ER localized zinc transporter ZIP7. CK2 phosphorylates ZIP7$^{Ser275/Ser276}$, which is a requirement for ZIP7-dependent cytoplasmic

release of zinc ions, suggesting that CK2 controls zinc gated release. Phosphorylation of ZIP7$^{Ser275/Ser276}$ is also required for activation of Akt and ERK1/2 following cell stimulation with zinc [193].

Table 1 – List of growth factor-dependent CK2 functions in the activity of growth and survival signaling pathways.

CK2 activity	Function
KSR interaction	Activation of MEK and ERK; Phosphorylation of B/C-Raf
B/C-Raf phosphorylation	Activation of ERK MAPK pathway
ERK phosphorylation	ERK nuclear translocation
MKP3 interaction	Decreased MKP3 phosphatase activity towards MEK
PAK1 phosphorylation	Activation of PAK1 monomers
α-catenin phosphorylation	Disruption of α-catenin-β-catenin interaction, induction of Wnt signaling
Dsh phosphorylation	Induction of Wnt signaling
β-catenin phosphorylation	Increased β-catenin stability and transcriptional activity
LEF phosphorylation	Decreased LEF-1 affinity for TLE1; increased affinity for β-catenin and Wnt target gene transcription
Survivin phosphorylation	Increased survivin anti-apoptotic and growth promoting activities
Tel2 and Tti1 phosphorylation	Increased mTORC stability; mTORC1 inhibition on growth factor withrawal
Akt interaction	Increased Akt activity
Akt phosphorylation	Increased Akt activity; Increased affinity for Hsp90 and stability
PTEN phosphorylation	Decreased PTEN phosphatase activity; increased stability; protection from caspase cleavage
NEP phosphorylation	Disruption of NEP-PTEN interaction, deficient PTEN recruitment to PM
IP6K2 phosphorylation	Decreased IP6K2 stability, reduced production of inositol pyrophosphates
ZIP7 phosphorylation	Zinc ion cytoplasmic release, activation of zinc signaling

Indirect regulation of PI(3)K/Akt signaling by CK2 is further exemplified by its interactions with the tumor-suppressor PTEN and PTEN-binding protein NEP. The phosphatase PTEN antagonizes PI(3)K/Akt signaling by dephosphorylating phosphatidylinositol-phosphates, such as PIP_2 and PIP_3, generated by PI(3)K at the plasma membrane. NEP is a metalloprotease that regulates the availability of peptides for surface-receptor binding, and anchors PTEN at the plasma membrane. Dephosphorylation of PIP_3 by NEP-bound PTEN at the plasma membrane prevents PIP_3-mediated activation of Akt thus suppressing cell survival. PTEN interacts with CK2 in cells, and is constitutively phosphorylated *in vivo* between C-terminal residues 369 and 386. Multiple groups have reported that CK2 phosphorylates PTEN at Ser370, Ser385, and Ser380/Thr382/Thr383 cluster *in vitro*, and is a strong candidate for phosphorylation of these residues in a physiological context. Mutation of Ser380, Thr382, or Thr383 to alanine decreases PTEN phosphatase activity, while increasing its stability

against proteasomal degradation and caspase cleavage. Mutation of all CK2 phosphosites in PTEN enhances these effects [194–198]. Furthermore, CK2 phosphorylation of $PTEN^{Ser370}$ primes $PTEN^{Thr366}$ phosphorylation by GSK3β, again illustrating the regulatory cooperativity between these two kinases [196].

NEP is a binding partner of CK2 and is able to phosphorylate the main NEP phosphosite *in vitro*. Putative CK2-dependent phosphorylation of NEP^{Ser6} inhibits PTEN-NEP interaction and subsequent PTEN recruitment to the plasma membrane, suppressing PTEN-dependent Akt inhibition [199]. The PD-1 receptor is an inhibitor of the PI(3)K/Akt pathway during activation of T cells in a PTEN-dependent manner. Following T cell-activating stimuli, PD-1 downregulates CK2 and potently inhibits CK2 activity, thus preventing CK2 phosphorylation of the PTEN at the Ser380/Thr382/Thr383 cluster. Concordant with the previous results, this led to an increase in PTEN phosphatase activity, but to a decrease in cellular stability [200]. Collectively, CK2 emerges as a potent modulator of PTEN cellular dynamics, further contributing to its wide role in promotion of Akt signaling.

Inositol pyrophosphates are also inhibitors of the Akt pathway by competing with PIP_3 for Akt binding. The pro-apoptotic kinase IP6K2 is one of the main enzymes responsible for inositol pyrophosphate synthesis and its deletion sensitizes cells to p53-mediated apoptosis. CK2 appears to be the physiological kinase of Ser347 and Ser356 in the PEST sequence of IP6K2. This phosphorylation enhances IP6K2 ubiquitination and subsequent degradation by the ubiquitin-proteasome system, as confirmed by the increased stability of a phosphoresistant $IP6K2^{Ser347/Ser356}$ mutant in cells. Thus, CK2 may promote cell survival by negatively regulating IP6K2 and preventing synthesis of Akt-inhibiting inositol pyrophosphates [201]. The results discussed in this section unarguably define CK2 as an important molecule in growth signal transmission and crosstalk. Some of the CK2 regulatory events that promote growth are listed in Table 1. Additional CK2 functions in survival and developmental signaling pathways such as JAK-STAT, JNK MAPK, Hedgehog, Urokinase, FGF, Notch, TGF-β, and Adiponectin have been described but will not be discussed here [202–206]. However, it is noteworthy that activation of many of these pathways leads to altered CK2 dynamics, further supporting the idea that CK2 acts in a context-dependent manner to coordinate cell behavior.

Programmed cell death

The pro-survival functions of CK2 do not rely exclusively in the stimulation of survival pathways, but also on direct regulation of apoptotic signaling pathways. Recent evidence indicates that CK2 suppresses both receptor-mediated and intrinsic apoptotic responses. In support, CK2 inhibition sensitizes tumor cells to both types of apoptotic induction [42, 207–211]. Overexpression of CK2α or CK2α/β, but not CK2β alone, is sufficient to protect cells from apoptosis after treatment with apoptosis-inducing agents such as etoposide and diethylstilbestrol [212, 213]. Moreover, CK2α/β overexpression enhanced apoptotic suppression when compared with CK2α alone. This suggests that CK2α is necessary for efficient suppression of apoptosis, and

CK2β potentiates this CK2α anti-apoptotic activity. Nuclear translocation of CK2 was detected after apoptotic stimuli and shown to be associated with protection from apoptosis [40–42, 46, 162, 210, 212, 214–218]. The anti-apoptotic parathyroid hormone-related protein (PTHrP) undergoes a similar nuclear translocation following apoptotic stimuli. Nuclear accumulation of PTHrP is sufficient to inhibit mitochondrial-induced apoptosis through regulation of CK2 activity, expression and subcellular localization [214]. Whether PTHrP is also able to regulate CK2 in the absence of apoptotic stimuli or CK2 cytoplasmic activities needs further investigation.

Activation of the caspase cascade in an essential step in apoptosis and is necessarily under tight regulation. Recently, CK2 emerged as a regulator of caspase proteolytic signaling at different levels. Direct binding with caspase inhibitors such as ARC prevents caspase activation. ARC is phosphorylated at Thr149 in a CK2-dependent manner in HEK293 cells, and phosphorylation of this residue is necessary for ARC anti-apoptotic activity. Furthermore, phosphorylation of ARC^{Thr149} is necessary for targeting ARC to mitochondria and for ARC-caspase-8 interaction, which suggests an important function for CK2 in regulating ARC activities [219]. FLIP is a caspase-like protein that lacks catalytic activity but competes with caspase-8 for presence in the pro-apoptotic DISC complex, inhibiting DISC activation in response to both TRAIL- and FasL-induced apoptosis. The expression and protein levels of FLIP are controlled by CK2 in carcinoma cells, suggesting that CK2 can control the cellular sensitivity to apoptotic induction by TRAIL and FasL [220]. Activation of the initiator caspase-2 depends on dimerization prior to proteolytic cleavage. CK2 phosphorylation of procaspase-2^{Ser157} prevents dimerization and maintains procaspase-2 inactive in the absence of apoptotic stimuli [221].

Prevention of caspase-mediated cleavage through phosphorylation of caspase recognition motifs is another mechanism through which CK2 protects cells from apoptosis. Proteolytic activation of procaspase-9 by caspase-8 is inhibited by CK2-mediated phosphorylation of caspase-9^{Ser348} [222]. CK2 also phosphorylates procaspase-3, which prevents its cleavage by caspase-8 and caspase-9 in HeLa cells [223]. Since procaspase-3 is the convergence point of both intrinsic and extrinsic apoptotic cascades, CK2 regulation could be crucial in the terminal decision to enter apoptosis. Phosphorylation of caspase recognition motifs by CK2 was reported to protect important pro-apoptotic and pro-survival proteins from caspase proteolytic cleavage, including Bid, Max, YY1, presenilin-2, HS1, connexin 45.6, and PTEN [195, 224–229]. Recently, an extensive overlap between CK2 phosphosites and capsase recognition motifs in the human proteome was detected with an *in silico* approach [223]. This overlap suggests that phosphorylation-dependent regulation of caspase activity by CK2 could be a general mechanism for apoptotic suppression. Collectively, CK2-mediated regulation of caspase inhibitors, caspase maturation, and caspase proteolytic activity, highlights the importance of the anti-apoptotic function of CK2 in the promotion of cell survival (Figure 7).

The tumor-suppressor PML is necessary for assembly of nuclear matrix-associated structures – PML nuclear bodies (NBs) – and controls key pathways for growth suppression, induction of apoptosis and senescence. $PML^{-/-}$ mice are susceptible to cancer development and aberrant degradation of PML is associated with tumor progression. It has been shown that CK2 regulates PML degradation in both physiological and oncogenic conditions [47]. CK2 phosphorylates PML^{Ser517}, and to a lesser extent $PML^{Ser512/Ser513/Ser514}$, promoting PML polyubiquitination and subsequent proteasomal degradation in mice fibroblasts. Impairment of CK2-mediated PML phosphorylation enhances the tumor suppressive activity of PML in Colo320DM human cells [47]. Since CK2 is frequently upregulated in tumors, PML increased turnover in these conditions might contribute to CK2-driven tumor development. Moreover, activation of p38-MAPK by different stress stimuli induced CK2-dependent degradation of PML, promoting a survival response [47]. Interestingly, CK2-dependent $PML^{Ser512/Ser513/Ser514}$ phosphorylation occurs in a SUMO interaction motif (SIM) and is necessary for PIAS1-mediated SUMOylation of PML [230, 231]. PML SUMOylation enhances the efficiency of CK2 phosphorylation of PML^{Ser517} [231]. Therefore, CK2 primes PML for PIAS1-mediated SUMOylation, which in turn primes CK2-dependent phosphorylation of PML^{Ser517}, indicating a dual self-potentiating inhibitory CK2 activity on PML protein levels. Supporting an important function for CK2 in PML regulation, the Epstein-Barr viral protein EBNA1 was shown to hijack the host CK2, which triggers CK2-dependent PML phosphorylation

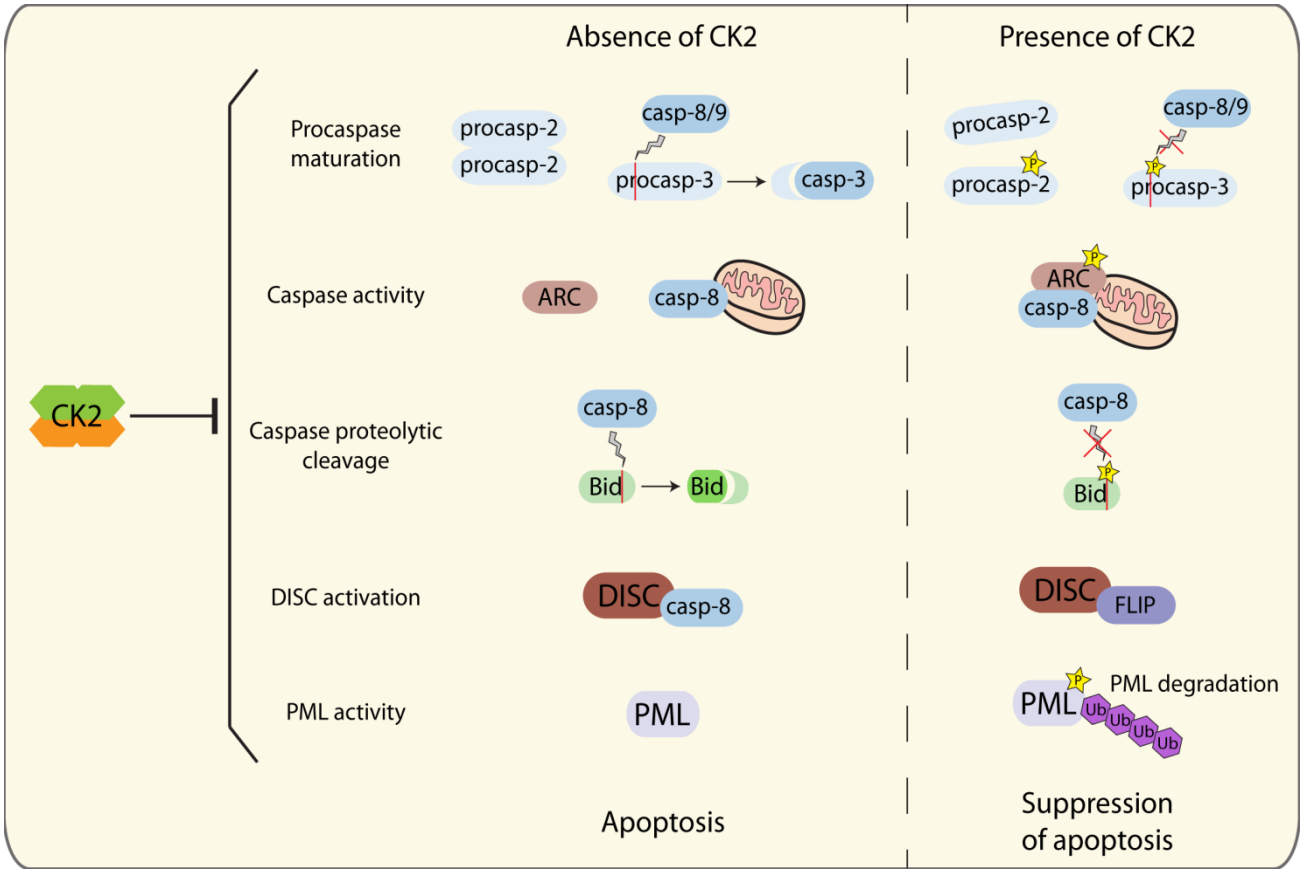

Figure 7 – Schematic representation of CK2 in apoptotic suppression. CK2 activity inhibits the apoptotic cascade at different levels and through distinct regulatory mechanisms. P – phosphorylation; Ub – ubiquitination; Bolt – caspase proteolytic activity.

and subsequent PML degradation [232]. Taken together, these data suggest that CK2 is a crucial regulator of PML activity in stressed and unstressed conditions, and that the CK2-PML axis is an important facet of both pro-survival and anti-apoptotic functions of CK2 (Figure 7).

Recent reports have also connected CK2 with regulation of bulk autophagy, an alternative pathway of programmed cell death [90, 233], but the molecular mechanisms involved remain unclear. In yeast, however, CK2 coordinates mitophagy, an autophagy-like process, by phosphorylating the mitochondrial outer membrane protein Atg32, which allows mitochondria to be targeted to the vacuole for degradation. This process was shown to be dependent on the yeast p38-MAPK ortholog Hog1 [234], highlighting the conservation and importance of the p38-MAPK-CK2 signaling axis.

Cell cycle

Progression through the cell cycle involves the timely activation of molecular signaling cascades that largely depend on phosphorylation and protein degradation events. A large body of evidence from multiple organisms demonstrates that CK2 is required for cell cycle progression. Experiments in yeast, *Drosophila*, and mammalian cells indicate that CK2 activity is necessary for the G_0/G_1, G_1/S, and G_2/M transitions as well as for proper mitotic progression [29, 158, 235, 236]. Genome-wide expression data indicate that approximately a quarter of the yeast genes regulated in a cell cycle-dependent manner are affected in strains lacking either catalytic or regulatory CK2 subunits [237]. Additionally, phosphoproteomic analysis in human cells indicates that a large portion of cell cycle physiological phosphosites are contained within a CK2 consensus site [7, 8]. In support for an active role throughout the cell cycle, drug-induced arrest at these transitions causes a reduction in nuclear matrix-associated CK2 with no apparent changes in whole-cell CK2 protein levels [238]. Mammalian CK2 kinase activity fluctuates during the cell cycle, and both CK2α and CK2β are phosphorylated in a cell-cycle dependent manner [153, 236, 239, 240]. The cyclin-dependent kinase Cdk1 efficiently phosphorylates CK2α and CK2β mitotic phosphosites *in vitro*, but has never been confirmed as a physiological CK2 regulator [241, 242]. The cell cycle dependent CK2α phosphorylation sites are not present in CK2α' although they are conserved between distantly related CK2α homologs [243]. This suggests functional specialization between the paralogous catalytic subunits, and might be the underlying reason why CK2α' homozygous knockout mice are viable while CK2α$^{-/-}$ are not

Cell cycle progression is driven by activation and inactivation cycles of cyclin-dependent kinases (Cdks). Among the factors controlling Cdk-dependent signaling is the Cdk-activating kinase (CAK) complex. In mammalian cells, the CAK complex and CK2 colocalize in the nucleus, and associate exclusively via a cyclin H-CK2α interaction [244]. Cyclin H^{Thr315} is a CK2 substrate *in vitro*, and phosphorylation of this residue is needed for full kinase activity of CAK [245]. Given that the CAK complex is known to promote activation of several Cdks, CK2 contribution to CAK activation might influence Cdk activity throughout the cell cycle. The CK2

putative regulator Cdk1 is a potent driver of both G_1/S and G_2/M transitions, and is phosphorylated at Ser39 specifically during the G_1 phase in mammalian cells [246]. This site is conserved in the budding yeast Cdk1 protein and both the yeast and human Cdk1^{Ser39} are efficiently phosphorylated by CK2 *in vitro*. Mutation of the putative CK2 phosphosite in Cdk1 causes a substantial decrease in both cellular volume and protein content, suggesting that CK2 contributes to Cdk1 regulation in the G_1 cell cycle phase [247].

Studies in yeast and mammalian cells suggest that CK2 has multiple functions in the G_1/S transition and contributes to the coordination of cell cycle events. In the budding yeast, correct entry into S phase requires degradation of the cyclin-dependent kinase inhibitor Sic1 at the end of the G_1 phase. Sic1 binds to CK2α and is phosphorylated by CK2 *in vivo*. Mutating the CK2 phosphosite abrogates the coordination between growth and cell cycle progression, indicating that CK2 phosphorylation regulates Sic1 cell cycle activities [248, 249]. Similarly, the human Sic1 ortholog, p27^{Kip1}, is phosphorylated by CK2 in cells in a CK2β-dependent manner [250, 251]. In cardiomyocytes, unphosphorylated p27^{Kip1} inhibits CK2α' activity, whereas growth factor-induced CK2α' phosphorylation of p27^{Kip1} impairs p27^{Kip1}-CK2 binding and targets p27^{Kip1} for degradation by the ubiquitin-proteasome system [251]. The E2 ubquitin-conjugating enzyme Cdc34 is required for ubiquitin-proteasomal degradation of important cell cycle regulators such as Sic1, Wee1, and IκBα. CK2-mediated multisite phosphorylation of yeast Cdc34 and human Cdc34/Ubc3 and Cdc34B/Ubc3B has been reported in several studies [252–255]**.** Phosphorylation of human Cdc34^{Ser231} and Cdc34B^{Ser233} by CK2 promotes their association with the β-TrCP subunit of the E3 ubiquitin ligase SCF, putatively influencing substrate recognition for the SCF complex [253]. In yeast, phosphorylation of Cdc34$^{Ser207/Ser216}$ was shown to induce Sic1 ubiquitination *in vitro*, and to be necessary for proper cell cycle progression and Sic1 degradation *in vivo* [255]. Moreover, CK2-dependent phosphorylation of Cdc34$^{Ser130/167}$ is required for complementation of a cdc34^{ts} mutant arresting at G_1 [256]. These results suggest that CK2 is a conserved regulator of Cdc34 catalytic activity and SCF affinity, which are required for G_1/S transition. The atypical protein kinase Rio1 is essential for ribosomal biogenesis, minichromosome maintenance, and cell cycle progression. In budding yeast cells, CK2 binds Rio1 and phosphorylates multiple sites in the Rio1 C-terminus, stimulating Rio1 kinase activity and its proteasome-mediated degradation during the G_1/S transition [257].

Entry into S phase in budding yeast depends on transcription of the G_1 regulon (start-specific transcription) that is involved in DNA synthesis, bud emergence and spindle pole body duplication. Transcription of the G_1 regulon depends on two transcription factor complexes SBF and MBF, which associate with transcriptional coactivators to recruit RNA polymerase II. A yeast strain lacking both CK2 regulatory subunits shows a delayed entry into S phase, reduced expression levels of G_1 regulon genes, and impaired MBF and SBF binding to G_1 regulon promoters. The common protein in both MBF and SBF complexes, Swi6/HP1, is phosphorylated by CK2 *in vivo***,** but no defect in cell cycle progression was observed when the CK2

phosphosite was mutated [258]. This suggests that CK2 is necessary for timely induction of start-specific transcription, although the molecular mechanisms behind this CK2 function remain unclear. CK2-dependent phosphorylation of Swi6/HP1 was previously shown to be necessary for genotoxic stress response activity of human HP1, and for its transcriptional gene silencing activities in fission yeast and *Drosophila* [106, 108, 109]. In these studies, different CK2 phosphosites were identified in Swi6/HP1, hence Swi6 start-specific transcription might depend on additional CK2 phosphorylation.

Histone synthesis is a cell cycle-regulated process and is at a maximum during S phase when the DNA is being replicated. Histone gene transcription during S phase of budding yeast cells was shown to be dependent on phase specific synergistic phosphorylation of the chromatin factor Yta7 by Cdk1 and CK2 [259]. Histone mRNA stability during S phase is maintained by the RNA stem-loop binding protein SLBP. Degradation of mammalian SLBP at the end of S phase is mediated by two phosphorylation events in which Cdk1 primes subsequent CK2 phosphorylation [260]. The chromatin factor Nap1 is necessary for nuclear import of histone H2A and H2B as well as cell cycle progression, and is a CK2 physiological target in budding yeast. Mutation of CK2 sites impairs Nap1 translocation to the nucleus and prolongs S phase, indicating that relevant Nap1 functions in the cell cycle are controlled by CK2 [261]. These results suggest that CK2 exerts multilevel control over histone expression and activity during the cell cycle, impacting genomic stability and cell cycle progression. Besides the putative Cdk1-mediated phosphorylation of CK2 subunits, Cdk1 activity as a CK2 priming kinase revealed a mechanism of CK2 temporal regulation and substrate-specificity during the cell cycle.

Similarly to its function during the G_1/S transition, growing evidence supports an important role for CK2 in the G_2/M transition and during mitosis. As previously mentioned, the G_2/S transition requires activation of Cdk1 signaling. Studies in mammalian cell cultures indicate that CK2 regulates the timing of Cdk1 mitotic activation in multiple ways. The Wee1 kinase is an inhibitory regulator of Cdk1 during the G_2 phase and its degradation via the ubiquitin-proteasome system is necessary for entry into mitosis. Efficient Wee1 degradation requires phosphorylation of $Wee1^{Ser123}$ by Cdk1, which primes subsequent phosphorylations by the polo-like kinase Plk1 and CK2. Phosphorylation of $Wee1^{Ser53/Ser121}$ by Plk1 and CK2, respectively, generates two binding sites for β-TrCP, a substrate recognition subunit of the E3 ubiquitin ligase SCF. Moreover, perturbation of the $Wee1^{Ser121}$ phosphorylation status delays Wee1 degradation and entry into mitosis [262]. Another study confirmed these observations, showing that CK2β siRNA depletion stabilizes the Wee1 protein, disrupts Plk1-Wee1 interaction, and impairs $Wee1^{Ser53/Ser121}$ phosphorylation [263]. These results indicate that CK2 activity is required for timely onset of mitosis by controlling Wee1. A parallel can be drawn between CK2 functions in the G_1/S and G_2/M transitions in which it regulates the degradation of Cdk1 inhibitors.

During the G_2/M transition, activation of Cdk1 further involves dephosphorylation of inhibitory Tyr residues by the closely related dual-specificity phosphatases CDC25B and CDC25C. CDC25B and CK2 associate in cells via CK2β. Moreover, CK2 catalyzes the phosphorylation of CDC25B$^{Ser186/187}$ *in vitro* and in cells, increasing its phosphatase activity [264]. CDC25C is also efficiently phosphorylated by CK2 *in vitro*. The putative CK2-dependent phosphorylation of CDC25C^{Thr236} negatively regulates its nuclear import *in vitro* and in HeLa cells [265]. Even though the cell cycle dynamics of CDC25B$^{Ser186/187}$ and CDC25C^{Thr236} phosphorylations were not analyzed, CK2 regulation of these phosphatases could contribute to cellular entry into mitosis.

Interactions with additional substrates suggest a function for CK2 in the regulation of chromatin structural dynamics during mitosis. Topoisomerases regulate DNA supercoiling by making DNA SSBs or DSBs, followed by condensation and relaxation of DNA strands. Topoisomerase II forms a stable complex with CK2 and is phosphorylated by this kinase *in vitro* [266]. Studies in budding yeast suggest that this interaction and phosphorylation occur and promote Topoisomerase II activity *in vivo* [267, 268]. In mammals, the physiological consequences of CK2-Topoisomerase II interaction are unclear since mutation of the CK2 phosphosites does not change Topoisomerase II activity [266]. However, CK2-mediated phosphorylation of human Topoisomerase I was also described *in vitro*, putatively enhancing Topoisomerase I DNA binding activity and relaxation of supercoiled DNA [269]. Condensin I is essential for assembly and maintenance of mitotic chromosomes, and strong evidence suggests that CK2-mediated phosphorylation of Condensin I occurs *in vivo.* It is known that in mammals Condensin I is phosphorylated by Cdk1 during mitosis, promoting its DNA supercoiling activity. In contrast, CK2-dependent phosphorylation of Condensin I was mostly detected during interphase, and suppresses Condensin I-mediated DNA supercoiling [270]. Given that CK2 has nuclear functions during mitosis, it is unclear at present how this temporal regulation of Condensin I is achieved. Nevertheless, these experiments suggest that CK2 activity can regulate the condensation state of mitotic chromatin through different substrates, putatively contributing to correct mitotic progression.

Additional mitotic functions have been described for CK2 in mitotic spindle assembly and subsequent chromosomal segregation and genomic stability. Depletion of either catalytic or regulatory CK2 subunits leads to centrosomal abnormalities and mitotic defects in budding and fission yeast, *Drosophila*, and mammalian cells [235, 271–273]. Furthermore, inhibition of CK2 or impairment of CK2α cell cycle-dependent phosphorylation leads to cell cycle progression in the presence of spindle defects in both fission yeast and mammalian cells [271, 272, 274]. This suggests that CK2 activity is necessary to activate the spindle assembly cell cycle checkpoint. In contrast, absence of CK2 in budding yeast led to the activation of the spindle assembly checkpoint, although the reason for this discrepancy is not yet known [273]. Immunolocalization and proteomic analysis have identified CK2 as a component of kinetochores and the mitotic spindle across all species examined [273–276].

Recently, relevant regulatory mechanisms controlling CK2 mitotic activity, as well as important CK2 functions at the kinetochore have been described. CK2α and CK2β are maximally phosphorylated during early mitosis, while phosphorylation decreases in anaphase, and is absent in telophase. CK2α mitotic phosphorylation is required for CK2 for CK2 localization to the mitotic spindle [274, 277]. In support of the preponderant role of specific CK2α phosphorylation during the cell cycle, CK2α' is not detected at mitotic spindles [275]. Moreover, expression of phosphomimetic CK2α leads to abnormal centrosomal amplification and chromosomal segregation, increasing catastrophe of mitotic cells. On the other hand, expression of a phosphoresistant CK2α abrogates drug-induced mitotic arrest, further suggesting a function of CK2 in the spindle assembly checkpoint [236]. An explanation for the importance of CK2α phosphorylation during mitosis comes from the observation that the phosphorylated form is required for CK2 binding to the peptidyl-prolyl *cis*/*trans* isomerase Pin1 in cells [274]. The Pin1 enzyme recognizes proline-directed phosphorylated Ser/Thr amino acid sequences, and alters the target protein by changing the peptide bond conformation preceding proline residues. Pin1 is conserved in eukaryotes and is essential for mitosis and mitotic progression [278]. Silencing of Pin1 via siRNA, and mutations inactivating either Pin1 catalytic or substrate recognition domains, abolish CK2 localization at the mitotic spindle. These results strongly suggest that Pin1 activity regulates CK2 mitotic dynamics in a phosphorylation-dependent manner [274].

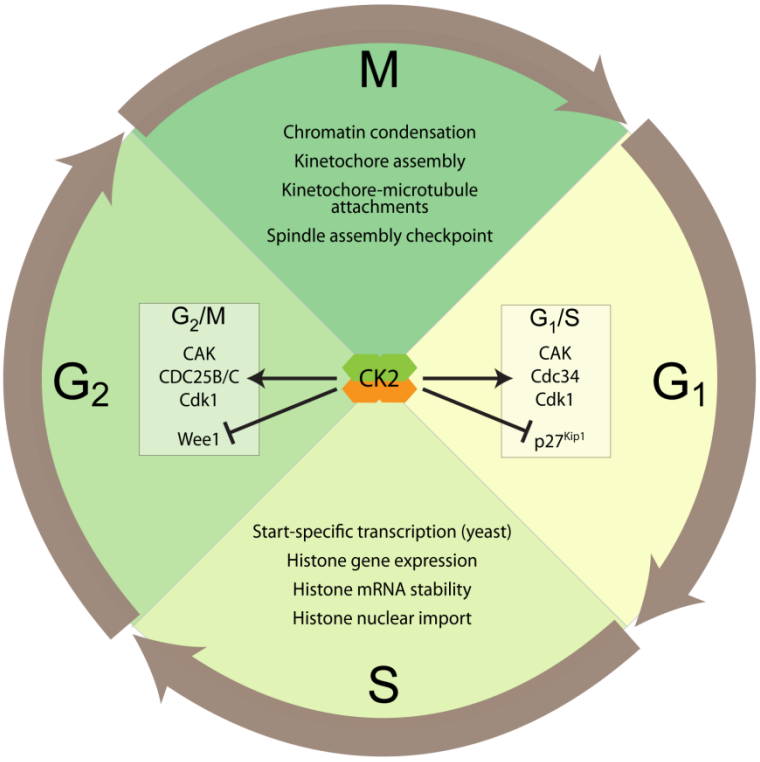

Figure 8 – CK2 activity in the coordination of cell cycle progression and cell cycle-specific chromatin dynamics.

Kinetochores are essential structures that coordinate chromosomal movements during mitosis by linking the centromeres to the mitotic spindle microtubules. Regulation of two important kinetochore components by CK2 was recently demonstrated during the budding yeast cell cycle. Ndc10 is part of the inner kinetochore, being essential for centromere-binding and for assembly of the entire kinetochore structure. Mif2/CENP-C is also necessary for kinetochore assembly and forms an important link between the inner and outer kinetochore. Ndc10 and Mif2 phosphorylation by the Aurora B kinase contributes to Ndc10 targeting to kinetochores, and is necessary for maintaining Mif2 protein levels and cell viability. CK2 also phosphorylates

both proteins, acting synergistically with Aurora B to properly localize Ndc10 at the centromere, and antagonizing Aurora B in the regulation of Mif2 stability [273]. In mammals, CK2 has been implicated in regulation of kinetochore-microtubule attachments through regulation of CLIP-170, a microtubule plus end-binding protein. CLIP-170 interacts with the dynein/dynactin complex to promote association of spindle microtubules with the kinetochore. CK2 appears to be the main kinase responsible for CLIP-170^{Ser1318} phosphorylation in cells, which promotes CLIP-170-dynactin interaction and CLIP-170 localization to the kinetochore. Moreover, CLIP-170^{Ser195} phosphorylation by Plk1 primes CK2-dependent CLIP-170^{Ser1318} phosphorylation, and both of these events contribute to timely formation of kinetochore-microtubule attachments in cells [279]. These results in both yeast and mammalian cells suggest a conserved function for CK2 in the regulation of kinetochore-associated functions that are essential for mitotic progression. Altogether, through the regulation of Cdks activity and chromatin dynamics, CK2 coordinates cell cycle progression and proper transmission of genetic material during cell division highlighting its fundamental role in eukaryotic organisms (Figure 8).

Expression and metabolism of gene products

A less understood and often dismissed aspect of CK2 cellular activity concerns the regulation of 'housekeeping' processes such as gene expression and gene product maturation. This should be fundamental to understand the physiological role of this pleiotropic protein, since it directly connects stimuli responsive CK2 to the regulation of cellular factors that mediate basic gene transcription, RNA processing, and protein maturation. Expression and maturation of gene products is a complex process that sustains cell growth and is regulated at multiple levels. Research done in multiple eukaryotes suggests an extensive contribution from CK2 activity in this process, although the data remains quite fragmentary. Described functions of CK2 in the regulation of the transcriptional machinery, RNA metabolism, and protein intracellular motility will be briefly summarized in this section.

Transcription

The initial steps in gene expression depend on the local chromatin state and the balance between transcriptional enhancers and repressors bound to promoter regions. As described in previous sections, CK2 directly regulates numerous chromatin remodeling factors (e.g. FACT, HP1, HDACs, or Condensin I), chromatin components (e.g. Histones), and transcription factors (e.g. NF-κB, p53, LEF, or Snail1). Although the regulatory functions of CK2 in chromatin remodeling will not be discussed further, CK2 has been found to regulate the activity of additional chromatin factors such as NCoR, SMRT, PRC1, Sin1, Brd4, Geminin, or Ikaros [280–286]. Binding of enhancers to promoter regions is followed by recruitment of the transcriptional machinery, which includes general transcription factors (GTFs) and the RNA polymerase (Pol) complex.

Eukaryotes have three distinct RNA Pols (I-III) that differ in the number and type of subunits, and that transcribe different classes of RNA molecules.

Genetic and biochemical evidence from yeast and mammalian cells suggests that CK2 functions as a regulator of the different transcriptional machineries. Pol I and Pol III are responsible for the transcription of structural and catalytic RNAs that function in RNA processing and protein synthesis. Transcriptional activity of Pol I and III varies widely depending on the metabolic state of the cell. Activity of Pol I and III is promoted in favorable metabolic conditions and is required for growth and proliferation, whereas stressing environments induce downregulation of Pol I and III-dependent transcription. Transcription of a mammalian Pol I target gene requires the formation of the pre-initiation complex (PIC) at promoter regions, which contains the RNA Pol and general transcription factors. The upstream binding factor (UBF) binds to Pol I-dependent promoters, and is responsible for PIC assembly through the UBF-SL1 interaction. SL1 is a complex composed of the TATA-binding protein (TBP) and three TPB-associated factors. UBF and SL1 synergize in the recruitment of Pol I and are necessary for transcriptional initiation, elongation, and reinitiation. Inhibition of CK2 reduces transcriptional activity of Pol I and limits transcription to a single round *in vitro* and in cells, suggesting that CK2 promotes Pol I-dependent transcription initiation and reinitiation [287–289]. CK2 associates with Pol I and is present in promoter regions of Pol I target genes in mammalian cells [287, 289]. It was shown that CK2 phosphorylates UBF in cells stabilizing the UBF-SL1 interaction, which induces transcriptional initiation and reactivation [287]. In contrast, phosphorylation of SL1 subunit TAF_I110 by CK2 destabilizes UBF promoter binding and prevents formation of the PIC complex [289]. SL1 and Pol I interact via the general transcription factor TIF-IA, which is also a putative CK2 substrate. CK2-mediated phosphorylation of TIF-IA releases TIF-IA from Pol I after initiation of transcription, and is necessary for Pol I-dependent transcription and cellular viability. Impairing TIF-IA phosphorylation by CK2 inhibits transcriptional elongation by Pol I [288]. Together, these results indicate that CK2 activity is necessary for efficient Pol I-dependent transcription by coordinating PIC complex assembly at the initiation and reinitiation stages. Moreover, by promoting TIF-IA dissociation from Pol I, CK2 regulates the switch from transcriptional initiation to elongation.

A similar function has been described for CK2 in the control of Pol III transcriptional activity. In both yeast and mammalian cells, CK2 associates with Pol III and can be seen at Pol III target promoters [290–293]. The core of the mammalian Pol III transcriptional machinery includes the TFIIIB and TFIIIC complexes. TFIIIC binds DNA at Pol III promoter regions and recruits TFIIIB and TFIIIB-associated Pol III in order to initiate transcription. CK2 is necessary for efficient Pol III-dependent transcription and is able to phosphorylate several Pol III accessory factors *in vitro* including Brf2, Bdp1, TBP, and $SNAP_c$ [292–295]. However, combined phosphorylation of TFIIIB components Brf2, Bdp1, and TBP *in vitro* inhibits transcription suggesting that CK2

can exert a dual effect on Pol III activity [293]. In support for a dual CK2 function, CK2 inhibition during mitosis restores transcription from mitotically inactive Pol III, whereas inhibition of CK2 during S phase debilitates transcription [294]. Cell cycle-dependent inhibition of Pol III transcription was shown to depend on mitosis-specific phosphorylation of Bdp1 by CK2 [294]. These data suggest that CK2 is a potent regulator of mammalian Pol III-dependent transcription, both by promoting Pol III activity during growth phases and by repressing it during mitosis.

Importantly, studies in yeast have revealed a direct connection between CK2 and the differential regulation of transcription in distinct environmental conditions. It was shown that CK2 is required for the activation of Pol III transcription following shift from restrictive to favorable growth conditions. This CK2 function depends on its catalytic activity through inhibitory phosphorylation of the conserved Pol III repressor Maf1 [291]. Although it is unclear how Maf1 phosphorylation by CK2 is inhibited in restrictive growth conditions, this function positions CK2 as a prime regulator of growth induction. CK2 was also described as a driver of Pol III transcriptional repression induced by genotoxic stress [290]. In unstressed yeast cells, CK2 associates with TBP via CK2β, and promotes TBP activation by phosphorylation of TBP^{Ser128}. Genotoxic stress caused by UV or MMS induces dissociation of the CK2 catalytic subunits from the CK2-TBP complex, which inhibits TBP transcription-promoting activity. Dissociation of CK2-TBP is required for DNA damage-induced Pol III transcriptional repression, and proceeds via a yet unknown mechanism [290, 296]. Collectively, these results strongly suggest a conserved function for CK2 in the promotion of Pol I and III transcription, thus increasing the cells non-coding RNA content and growth potential. Furthermore, regulatory interactions with yeast Maf1, TBP, and human Bdp1 reveal a direct link between CK2 stress- and cell cycle-induced dynamics and modulation of Pol III transcriptional activity that differentially drives the cell towards growth or survival.

Pol II is responsible for transcription of protein-coding mRNAs and small regulatory RNAs. CK2 is present at promoters of Pol II target genes and associates with the Pol II transcriptional machinery in mammalian cells [297]. CK2β interacts with Pol II, TFIIA, TFIIF, and TBP *in vitro*. Phosphorylation assays *in vitro* suggest that CK2 has numerous substrates within the Pol II transcriptional machinery, including subunits of TFIIA, TFIIE, TFIIF and Pol II. Moreover, combined phosphorylation of TFIIA, TFIIF, and Pol II subunits by CK2 enhances Pol II transcriptional activity *in vitro* [298]. These findings suggest a role for CK2 in Pol II-dependent transcription although this has not been studied in detail. One aspect is which CK2 might contribute to Pol II activity is the regulation of Pol II affinity for different types of promoter elements [297]. It was shown that CK2 can abolish Pol II-dependent transcription from downstream core element (DCE) sequences in crude nuclear extracts of mammalian cells. In the same assay, CK2 was necessary for recognition of downstream promoter element (DPE) sequences by Pol II, suggesting that CK2 promotes transcription from DPE in detriment of DCE

elements [297]. Even though CK2 is detected in DPE and not DCE promoter elements in cells, the relevance of these findings needs further validation.

RNA metabolism

Transcription yields different types of RNA molecules that can be protein-coding mRNAs or non-coding RNA molecules that serve catalytic, regulatory, and structural functions. There are several indications that CK2 is a regulator of protein-coding and non-coding RNA metabolism. In yeast, CK2 was identified as a component of the pre-60S ribosomal RNA (rRNA) particle and of the small-subunit processome, a pre-ribosomal complex responsible for biogenesis of the 18S rRNA [299, 300]. In mammalian cells, nuclear CK2 is associated with the nucleolus, a nuclear structure where rRNA is transcribed and pre-ribosomal particles are assembled. Amongst CK2 *in vitro* substrates are important proteins for nucleolar structure such as B23/nucleophosmin and Nopp140 [301, 302]. In support of a nucleolar function for CK2, its inhibition induces nucleolar disorganization in cells. Moreover, CK2 inhibition or impairment of CK2-dependent phosphorylation of B23 leads to nucleolar reorganization in an *in vitro* assay [303]. Although inconclusive, these results support a putative function for CK2 in nucleolar function and ribosome biogenesis.

Recent studies suggest that CK2 might also be involved in the regulation of pre-mRNA splicing and mRNA stability. The *Drosophila* splicing factor PSI is phosphorylated by CK2, which allows PSI to interact with the RNA exonuclease Rrp1 [304]. In mammalian cells, CK2 co-localizes with the spliceosome and interacts with splicing factors such as Prp3p, 61K, and RNPS1. Prp3p and RNPS1 are also CK2 substrates, and phosphorylation of these factors at CK2 phosphosites was found to promote mRNA splicing. Even though the physiological relevance of these interactions remains unclear, they further attest to the wide extent of CK2 cellular functions [305–307]. Localized mRNA translation is a regulatory mechanism that plays an important role in polarity establishment and requires the transport and stability of mRNAs to the presumed place of translation. In budding yeast, CK2 assures localized transcription of the *ASH1* mRNA at the bud cortex through phosphorylation of the RNA-binding Puf6p protein, which promotes transport and stability of the *ASH1* transcript [308]. Similarly, CK2 phosphorylates the *Drosophila* CPEB protein Orb, which is required for localized translation of *gurken* mRNA during the oocyte dorso-ventral patterning [309]. These findings provide *in vivo* evidence for CK2 regulation of mRNA stability and translation, and another mechanism through which CK2 can drive cellular polarization.

Inhibition of CK2 impairs activity of the nonsense-mediated mRNA decay (NMD) mechanism, which degrades mRNAs with premature stop codons and other types of RNA molecules. In mammals, this mechanism relies on the activity of the PIKK kinase SMG1, the RNA-binding protein UPF1, and other molecules of the SURF complex. It was previously mentioned that SMG1 is stabilized in cells via CK2-mediated constitutive phosphorylation of Tel2$^{Ser487/Ser491}$ [188]. Phosphorylation of Tel2$^{Ser487/Ser491}$ was recently shown to enhance

UPF1 binding to target RNAs and UPF1-SMG1 interaction *in vitro* [310]. These results suggest that constitutive phosphorylation of Tel2 by CK2 might be a way to sustain continued NMD-mediated surveillance and degradation of aberrant RNAs.

Regulation of the human eukaryotic translation initiation factor 5 (eIF5) by CK2 provides strong evidence for a function of this kinase in global regulation of protein translation. eIF5 plays an important role in the initiation of translation since its GTP hydrolase activity is required for assembly of 80S ribosomal particles. It was show that growth factor stimulation enhances CK2 activity and promotes phosphorylation of $eIF5^{Ser389/390}$ by CK2. Depletion of CK2 or expression of a phosphoresistant $eIF5^{Ser389/390}$ mutant prevents efficient formation of the eIF5/eIF2/eIF3 translation initiation complex and impairs growth and cell cycle progression [157]. Regulation of eIF5 phosphorylation status by CK2 putatively reveals a pathway for modulation of translation levels in different developmental or environmental contexts.

Protein maturation

Protein activity and function are greatly influenced by their subcellular localization and by their ability to relocalize within the cell. A considerable portion of intracellular protein transport occurs via vesicle trafficking that connects the endomembrane system and depends on the dynamics and motor proteins of the cytoskeleton. A parallel system through which proteins can change their subcellular localization consists of import/export translocation machineries present in different organelles. Malfunctioning of either system can dramatically impair cellular behavior since putatively important proteins might be absent from their functional subcellular domains or wrongly localized. Data from recent studies suggests that CK2 has important regulatory functions in cytoskeleton reorganization, vesicle trafficking, and protein import/export.

Microtubule and filamentous actin networks are important components of the cytoskeleton, which, among other functions, serve as a platform for intracellular transport. As previously mentioned, CK2 is a MAP that binds microtubules and stabilizes microtubule networks [133, 141, 311, 312]. In support of these conclusions, siRNA depletion of CK2 in different human cell lines destabilizes the microtubule network, and sensitizes microtubules to the action of colchicine. The MAP function of CK2 is independent of its kinase activity and requires the tetrameric holoenzyme [133]. CK2 also interacts with other MAPs in cells such as MAP1B, the end-binding protein EB2, and the kinesin KIF5 [138, 313, 314]. The physiological relevance of CK2 as a MAP or as a MAP effector is still unclear, although a recent study revealed an interesting function for the CK2-KIF5 interaction. KIF5 can auto-inhibit its motor protein function and association with cargo through a head-tail interaction. It was shown that CK2 dose-dependently activates KIF5 *in vitro*, through a mechanism that does not require phosphorylation of KIF5 by CK2 and is independent of the KIF5 head-tail auto-inhibition. In support, reduction of CK2 levels but not inhibition of its kinase activity leads to a decrease in the activity of KIF5 motors in mammalian cells [141]. Since CK2 levels increase in actively growing cells,

this CK2-dependent upregulation of kinesin-based transport could be a mechanism through which cells potentiate cellular trafficking and secretion during growth.

Interestingly, CK2 was also shown to be an actin-binding protein *in vivo*. Assays conducted *in vitro* revealed that actin inhibits CK2α and CK2 catalytic activities in a dose-dependent manner through interaction with CK2α [315]. Several regulators of actin dynamics were described as CK2 substrates *in vitro,* but in most cases the biological relevance of the interactions was not tested (reviewed in [128]). Nonetheless, the described CK2-mediated regulation of the actin organizers coronin 2 (CRN2), WASP, and bacterial ActA provides strong evidence for an important CK2 function in actin organization. Phosphorylation of CRN2^{Ser463}, WASP$^{Ser483/Ser484}$, and ActA$^{Ser155/Ser157}$ by CK2 was shown *in vitro* and in cell cultures. [316–318]. The phosphorylated form of CRN2^{Ser463} is unable to inhibit actin polymerization in contrast with the CRN2 non-phosphorylated form. Phosphorylated CRN2^{Ser463} also shows impaired F-actin cross-linking activity and interaction with the Arp2/3 actin nucleation complex *in vitro*. In tumor cells, expression of a phosphomimetic form CRN2^{Ser463} alters the F-actin network organization and inhibits cell migration [316].

WASP/WAVE proteins regulate many functions that require reorganization of the actin cytoskeleton such as endocytosis, polarization, or cell migration. These proteins promote nucleation of actin through their VCA domain, which binds both actin monomers and the Arp2/3 complex. The CK2 phosphosites Ser483 and Ser484 are within the WASP VCA domain and are constitutively phosphorylated *in vivo*. Moreover, WASP$^{Ser483/Ser484}$ phosphorylation drastically enhances WASP affinity for the Arp2/3 complex in cell lysates, and is required for efficient WASP actin nucleation activity in assays with cell extracts [317]. ActA is a protein from the intracellular parasite *Listeria monocytogenes* that has a VCA-like domain and is required for parasitic motility. CK2 was identified in a screen for host factors that compromised *Listeria*'s ability to spread from cell to cell, which requires the nucleation of an actin tail [319]. Presence of host CK2 is required for efficient tail formation in *Listeria* infected cells. Similarly to WASP, the CK2 phosphosites Ser155 and Ser157 are within the ActA VCA-like domain and are required for efficient Act binding to Arp2/3. The significance of these phosphorylations was tested *in vivo* using bacteria mutated at the *actA* locus that coded for a phosphoresistant ActA$^{Ser155/Ser157}$ mutant protein. The introduction of this double mutation in ActA led to severe defects in actin tail formation and *Listeria* cell-to-cell spread, suggesting a pivotal function for CK2 in determining the pathogenic potential of *Listeria* [318]. These results also suggest a putative regulatory mechanism in which VCA phosphorylation by CK2 can dynamically regulate WAVE/WASP-dependent actin-based processes in different cellular contexts.

Vesicle trafficking connects the endomembrane system and is responsible for delivery of molecular cargo to specific compartments. COPII-coated vesicles mediate export of cargo from the ER to the Golgi network. Formation of COPII-coated vesicles depends on the small GTPase Sar1 and six Sec proteins. Sar1 is activated

by the ER-localized guanine exchange factor Sec12, leading to the recruitment of the Sec23-Sec24 heterodimer. Sec23 in turn recruits the Sec31-Sec13 heterodimer, which promotes budding of COPII-coated vesicles from the ER. CK2 was shown to phosphorylate Sec31 at multiple residues resulting in decreased Sec31 association with the ER membrane and binding to Sec23. Furthermore, both CK2 depletion by siRNA or expression of a phosphomimetic Sec31 mutant at CK2 phosphosites dramatically reduced ER-to-Golgi transport in mammalian cells [320]. These results suggest that CK2 activity might promote budding of COPII-coated vesicles by reducing the affinity of Sec31 towards Sec23. Since protein export from the ER is a rate-limiting step of molecular trafficking and secretion, this activity of CK2 again highlights its importance in the regulation of crucial systems required for growth.

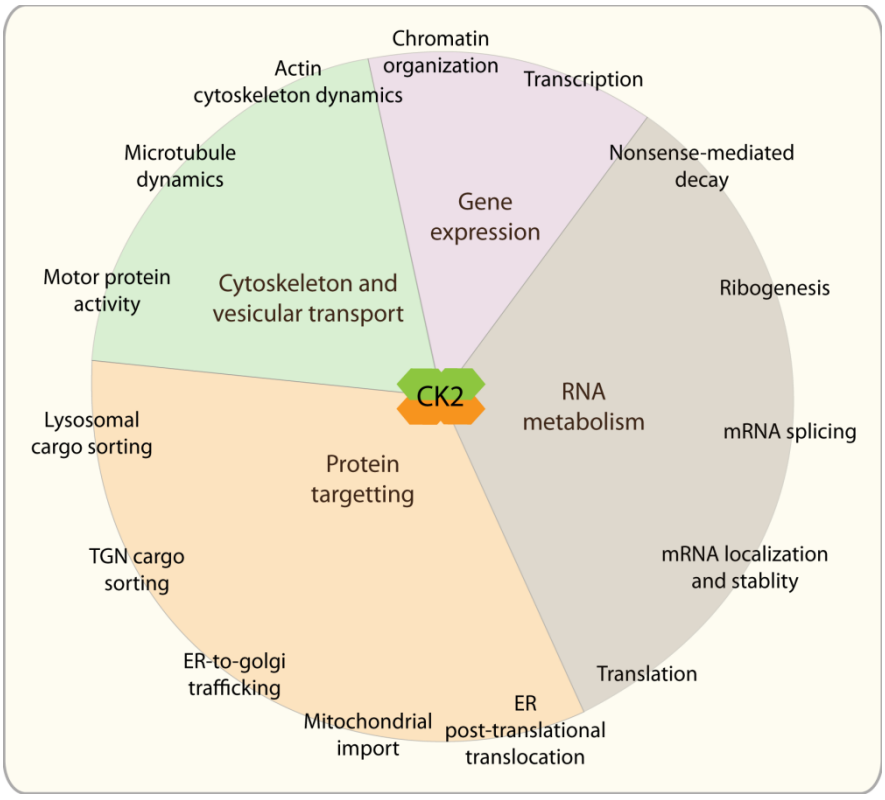

Figure 9 – Eukaryotic processes necessary for cell growth and activity regulated by CK2.

The cation-independent mannose-6-phosphate receptor (CI-MPR) follows a highly regulated itinerary to deliver hydrolases that mature in the Golgi network to lysosomes. CK2 was shown to play an important regulatory function in the differential targeting of CI-MPR through phosphorylation of distinct cargo adaptors of the GGA and PACS families [321, 322]. CK2 has also been shown to influence protein sorting to specific compartments by directly phosphorylating the cargo protein. This is the case for sorting of the cation channel polycistin 2 (TRPP2), in which phosphorylation of an acidic cluster by CK2 modulates the binding affinity of different cargo adaptor molecules of the PACS family that mediate trafficking to distinct compartments [323]. PACS proteins mediate cargo sorting by recognition of a phosphorylated acidic cluster. Since this acidic cluster is present in many membrane proteins transiting in the secretory pathway, the acidophilic CK2 might have a wider influence in protein subcellular targeting than what is currently known.

ER-resident proteins, proteins destined to other compartments of the endomembrane system, or secreted proteins undergo post-translational translocation into the ER. The protein Sec63 is an essential and conserved component of the ER import apparatus. Mammalian Sec63 interacts with and is a substrate for CK2 *in vitro* [324]. This interaction has been studied in more detail in budding yeast. In this model, Sec63 is constitutively phosphorylated by CK2 *in vivo*, and phosphorylation of Sec63 is required for stability of the Sec63-Sec62 complex and efficient protein translocation into the ER [325]. Similarly, CK2 was shown to constitutively phosphorylate the mitochondrial import proteins Tom22 and Mim1 in yeast [326]. Tom22 is an essential component of the transporter outer membrane (TOM) mitochondrial translocation machinery, and Mim1 is necessary for TOM biogenesis and for an alternative mitochondrial import pathway. Phosphorylation of both Tom22 and Mim1 by CK2 is necessary for proper biogenesis of the TOM complex, thus protein import into mitochondria [326]. In conclusion, the functions described for CK2 throughout this section place CK2 as an important modulator of the activity of conserved eukaryotic machineries that are fundamental for gene product expression and maturation, hence globally regulating cellular function and growth (Figure 9).

Conclusions and outlook

The collective knowledge here described provides overwhelming evidence for a function of CK2 as a global regulator of cell behavior, whose activity is dependent on the cellular conditions and acts to coordinate and drive cellular change. The physiological consequences of altering CK2 expression or activity, and the function of CK2 regulatory targets unmistakably establish it as a pivotal factor for eukaryotic life. The remarkable number of described binding partners and substrates further attests to the importance of CK2, putatively the most pleiotropic protein kinase known. Unlike any other regulatory protein, the activity of CK2 is not restricted to favorable growth conditions or to the transduction of particular stimuli. Instead, the ubiquitous and constitutive CK2 is permanently active in the regulation of numerous fundamental cellular processes and signaling pathways. A shift in cellular conditions (e.g. caused by growth factor stimulation/withdrawal or UV exposure) and the subsequent activation of condition-specific signaling pathways induce changes in CK2 dynamics by altering the availability of CK2 substrates and regulators. Condition-specific modulation of CK2 dynamics seems to rely mostly on the regulation of its catalytic activity, substrate specificity, and subunit subcellular localization, and seldom acting at the level of gene expression or protein stability. Importantly, since CK2 activity regulates numerous signal transduction pathways and essential cellular processes, changes in CK2 dynamics tend to trigger profound modifications in cell behavior. This pleiotropy, together with the ubiquitous, essential, and constitutive nature of CK2 inevitably suggest a function as a global coordinator of cellular processes (Figure 10). Most notably, CK2 appears as a multifunctional promoter of cell growth when conditions are favorable, and of survival and adaptation during stress response. Nevertheless, supporting the

hypothesis that CK2 functions are shaped 'indiscriminately' by the specific context of a cell, CK2 activity is determinant in cell fate decisions besides growth and survival, as for example differentiation, migration, and polarity establishment. Interestingly, since CK2 dynamics are conditioned by most of the tested cell behavior-modifying stimuli and conditions, it is tentative to suggest that CK2 dynamics serve both as a permanent read-out of the conditions of the cell and as the driver of the appropriate cellular changes.

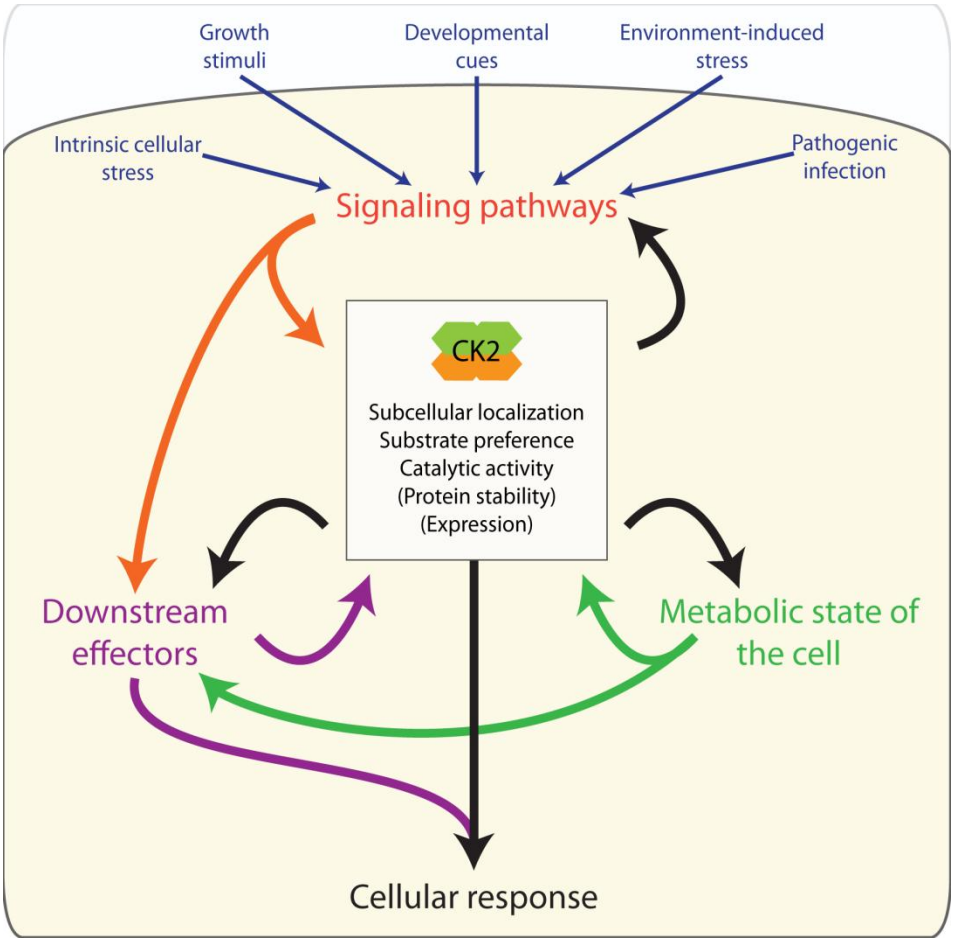

Figure 10 – Simplified model of CK2 function as a coordinator of cell change. The constitutive and pleiotropic CK2 permanently regulates the activity of numerous eukaryotic mechanism required for cell function and change. Activation of signaling pathways and downstream effectors or changes in cellular metabolism alter CK2 dynamics in a condition specific manner leading to distinct CK2-driven cellular responses.

The pleiotropy of CK2 functions, as well as the spatiotemporal and protein-interaction dynamics that regulate the activities of CK2 subunits raises an interesting question as to why the CK2α and CK2β families did not radiate like other multifunctional protein families. A possible explanation might stem from the fact that the balance of individual CK2 subunits determines CK2 activity, thus influencing cell fate. Consequentially, an imbalance in CK2 subunits concentration caused by increased gene dosage could have highly detrimental effects on fitness. Alternatively, clustering all these functions in a single enzyme might simply provide the most efficient and coordinated cellular response to CK2 inducing stimuli.

The paradox of a ubiquitous and constitutive enzyme that regulates spatiotemporally restricted processes is starting to fade with the elucidation of several regulatory mechanisms responsible for CK2 dynamics. Stimuli-activated binding partners seem to play a major role in determining the subcellular localization, catalytic activity, and substrate preference of CK2 in different conditions. An illustrative example is the EGF-induced

phosphorylation, nuclear translocation, and increased catalytic activity of CK2 that determine specific CK2 functions such as ERK2 nuclear shuttling, inhibition of MPK3, and activation of PAK1, B/C-Raf, zinc signaling, and the Wnt/β-catenin pathway. Another example could be the cell cycle-dependent Pin1 recognition and targeting of phosphorylated CK2α to the mitotic spindle where it performs diverse functions. Hierarchical phosphorylation events either as a priming or primed kinase constitute another preponderant regulatory mechanism that contributes to restrict the range of CK2 activities. An interesting consequence arising from this is that CK2 phosphoacceptor sites may be even more predominant than estimated using protein sequence scanning algorithms that do not consider the presence of *in vivo* phosphosites. Even though the essentiality of the CK2 enzyme is unquestionable, many of its regulatory functions are performed redundantly or synergistically by additional kinases. This could be another way to control an enzyme with the unusual properties of CK2, putatively underlying signal reinforcement and integration with other signaling pathways. Notwithstanding our current knowledge on how control over a constitutive kinase can be achieved in cells, the molecular details behind most condition-specific changes in CK2 dynamics are still unknown.

Although the analysis of a multitude of substrates identified in the last decades provides clues as to how CK2 acts in cells, postulation of functional models of CK2 activity and elucidation of specific CK2 functions remain extremely challenging tasks. Challenges reside in the essentiality and pleiotropy of CK2, which make it difficult to study with conventional approaches and establish direct causal relationships. Additionally, providing conclusive evidence for a direct kinase-substrate interaction is a notoriously difficult endeavor. The main challenge, however, is probably the complexity of CK2 activities. CK2 is differentially regulated in response to distinct stimuli, leading to complex rearrangements in subcellular distribution and activity of CK2 subunits. CK2 regulates and is regulated by numerous pathways serving as a global cross-regulatory molecule in the extensively interconnected cellular signaling network. More often than not, CK2 activity modulates the activity of a pathway at multiple levels, regulating both inducers and suppressors, and leading to different outcomes in distinct conditions. Considering as well that CK2 dynamics are locally defined by the balance between its subunits, a clear understanding of the function of CK2 in eukaryotic cells will probably have to rely on computational approaches.

Hindering the study of CK2 activity is also the lack of apparent similarities between its substrates. In common, they are highly conserved eukaryotic proteins, either involved in regulatory networks (e.g. receptors, scaffolds, kinases, transcription factors), or cellular processes that require constitutive activation to allow for growth and maintenance of cellular integrity (e.g. transcription, translation, vesicle trafficking, protein translocation). Nevertheless, relationships between CK2 and some widespread protein domains that are regulated by phosphorylation events have been described. These include CK2-mediated phosphorylation

of PEST sequences, Ankyrin binding motifs, sumo interacting motifs, caspase cleavage sites, ion receptor acidic clusters, or creation of FHA-domain recognition sequences. Whether these are general regulatory mechanisms employed by CK2, as opposed to isolated events, awaits further validation.

Another future challenge will consist of validating the biological significance of CK2 functions described throughout the years *in vitro* or in cell culture. In many cases, a physiological validation of functions described for CK2 was either not performed, or was plagued by the use of non-specific inhibitors such as DMAT and TBB. Most of the *in vivo* information on CK2 was acquired using yeast cells and *Drosophila*, while practically no studies were performed using the nematode *Caenorhabditis elegans*. The almost unmatched amenability of *C. elegans* to genetic and functional manipulation as well as the possibility of conducting high-throughput experiments *in vivo*, should position *C. elegans* as a preferential model in which to dissect CK2 function. Future studies on CK2 activity and regulation promise to deepen the scientific understanding on how cells initiate, coordinate, and sustain global responses to their environment. Recent technical development such as highly specific CK2α pharmacological inhibitors, dominant negative CK2α mutants, or conditional CK2 knockout mice will facilitate this elucidation process [104, 327]. Engineering of a CK2α ATP-binding cleft mutants that bind specifically to modified substrates and inhibitors, a strategy developed by Shokat and co-workers [328], would be a powerful tool to investigate physiological relevance of CK2-substrate interactions. Given the often antagonistic role of individual CK2 subunits and the pleiotropy of CK2, large-scale analysis of CK2 holoenzyme and subunit-specific interactions, targets, and localization in cells exposed to different stimuli should greatly contribute to this effort.

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