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14-3-3 Proteins As Signaling Integration Points for Cell Cycle Control and Apoptosis

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Abstract

14-3-3 proteins play critical roles in the regulation of cell fate through phospho-dependent binding to a large number of intracellular proteins that are targeted by various classes of protein kinases. 14-3-3 proteins play particularly important roles in coordinating progression of cells through the cell cycle, regulating their response to DNA damage, and influencing life-death decisions following internal injury or external cytokine-mediated cues. This review focuses on 14-3-3-dependent pathways that control cell cycle arrest and recovery, and the influence of 14-3-3 on the apoptotic machinery at multiple levels of regulation. Recognition of 14-3-3 proteins as signaling integrators that connect protein kinase signaling pathways to resulting cellular phenotypes, and their exquisite control through feedforward and feedback loops, identifies new drug targets for human disease, and highlights the emerging importance of using systems-based approaches to understand signal transduction events at the network biology level.

Keywords

14-3-3; DNA damage; cell cycle checkpoint; apoptosis; signal transduction; mitosis

1. Introduction

14-3-3 proteins bind to their phospho-Serine and phospho-Threonine-containing ligands to regulate a wide range of cellular phenomena. Many 14-3-3-binding proteins contain sequences that match one of two general consensus motifs: **RSx[pS/pT]xP** and **Rxxx[pS/pT]xP**, which are recognized by all 14-3-3 isotypes [1]. The Pro in the pS/pT+2 position, while preferred, is not absolutely required [1–3], particularly if the motif is found at the C-terminus [4]. Two of the most well established roles for 14-3-3 proteins are in the control of cell cycle progression and the regulation of apoptosis, and these remain areas of active ongoing research. In this article we briefly summarize the current status of research in these areas, focusing on a selection of the most recent papers that illustrate new concepts or expand on previously established models.

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2. 14-3-3 proteins in cell cycle control

2.1 The G2/M Checkpoint

Early studies implicated 14-3-3 as a critical integration point for many of the protein kinases and phosphatases that control the transition from G2 into M phase (Figure 1A). Normal mitotic entry from G2 is triggered by a rapidly enhanced activation of Cdk1/cyclin B (c.f. [5] and references therein). Cdk1 is activated by constitutive phosphorylation of a threonine residue (T161) within the “T-loop” by CAK (Cdc2-activating kinase) but maintained in an inhibited state by phosphorylation of a tyrosine residue (Y15) within the ATP-binding “P-loop” by the tyrosine kinase Wee-1 [5]. In addition, higher eukaryotes also have a membrane-bound kinase, Myt1, that phosphorylates both Y15 and the preceding threonine, T14, to ensure Cdk1 inhibition [5]. Removal of these inhibitory P-loop phosphorylations on Cdk1 to promote entry into mitosis from G2 is achieved by the Cdc25 family of dual-specificity protein phosphatases [6, 7]. Mammalian cells express three different Cdc25 proteins (A, B, and C), all of which have been implicated in G2/M progression. At the G2/M boundary, Cdc25B appears to be the trigger phosphatase that initially activates Cdk1/cyclin B, which in turn phosphorylates Cdc25C to create a binding site for a second mitotic kinase, Plk1 [8, 9], which in turn further phosphorylates Cdc25C [10]. This cascade of sequential phosphorylation events results in an autoactivation loop that subsequently drives entry into mitosis [7, 11–13]. While Cdc25A is predominantly recognized for its involvement in earlier phases of cell cycle progression (see below), it has been shown to also play a role at the G2/M transition [14, 15] and is the only Cdc25 family member that has an embryonic lethal phenotype in knockout mice [16]. The relative importance of Cdc25A in G2/M progression in normal cells probably depends on the status of Cdc25B and C, which may be influenced by the prior history of the cells.

Like Cdk1/cyclin B, the Cdc25 phosphatases are themselves regulated through activating and inhibitory phosphorylations. For Cdc25B and C, activating phosphorylations are generated by Cdk1 and Plk1 [10, 17–19] through the positive feedback loop described above, although data in *Xenopus* extracts suggests that MAPKs (particularly Erk1/2) may play a major role [20]. Whether this is also true in mammalian cells is less clear. Inhibitory phosphorylation events during G2 probably arise from the detection of ongoing endogenous DNA damage from normal replication events during the preceding S-phase, or exogenous DNA damage that the cell has encountered during late S and G2, and are mediated by three DNA damage-responsive kinases, Chk1, Chk2, and MK2 [21, 22] (see below). The main mechanism by which these inhibitory phosphorylations block Cdc25B and C function is through the phosphorylation-dependent binding to 14-3-3. Phosphorylation of a single binding site on Cdc25B (S323 in human), or Cdc25C (S216 in human) [23, 24] is necessary and sufficient for 14-3-3 binding, although there are some indications that additional binding sites may also contribute to their regulation [25, 26] in ways that are somewhat mechanistically unclear.

There are likely to be multiple mechanisms through which 14-3-3 proteins inhibit Cdc25B/C function. First, 14-3-3 binding to *Xenopus* Cdc25C causes a direct two-fold reduction in its phosphatase activity when assayed *in vitro* using Myt1-phosphorylated ³²P-labelled Cdk1 as a substrate [23]. A similar direct inhibition of Cdc25B catalytic activity is likely to occur based on pull-down data indicating that 14-3-3 binding to Cdc25B blocks Cdc25B binding to CyclinB/Cdk1 [25]. Second, 14-3-3 binding leads to the cytoplasmic sequestration of Cdc25B/C [27–30], likely blocking access to its nuclear substrate Cdk1/cyclin B [31], required for mitotic entry. The mechanism by which 14-3-3 proteins regulate the subcellular localization of their bound targets involves the selective exposure or shielding of specific sequence elements. This has been most clearly experimentally elucidated for Cdc25C, although the identical mechanism almost certainly functions for Cdc25B. In between the N-

terminal regulatory domain and the C-terminal catalytic domain of Cdc25C lies an intrinsic nuclear localization sequence (NLS) and a nuclear export sequence (NES) [28, 29]. These subcellular localization signals allow Cdc25C to shuttle between the nuclear and cytoplasmic compartments. The major 14-3-3 binding site in human and *Xenopus* Cdc25C (S216 and S287, respectively) is located right next to the NLS [28, 32], and in *Xenopus*, 14-3-3 binding to Cdc25C has been shown to block the binding of Cdc25C to the importin- α nuclear import receptor [29]. Thus, 14-3-3-binding causes defective nuclear import in the face of unaffected nuclear export [33], relocalizing most of Cdc25C to the cytoplasm.

Although all of the 14-3-3 isoforms except 14-3-3 σ can bind to Cdc25 isoforms in vitro [34, 35], it appears, on the basis of co-IP experiments using tagged overexpressed proteins that the ϵ and γ isoforms of 14-3-3 bind strongest to Cdc25C in cells [34], while yeast 2-hybrid experiments suggest that 14-3-3 β , η , τ , and ζ bind to Cdc25B [36]. Whether this marked 14-3-3 isoform exclusivity for the mitotic 'initiator' Cdc25B phosphatase versus the mitotic 'sustainer' Cdc25C phosphatase is biologically important remains unknown.

In addition to inhibiting the Cdc25 phosphatases that activate Cdk1, 14-3-3 proteins also bind to Wee1, the protein kinase that inhibits Cdk1. In this case, however, 14-3-3 binding serves to distribute Wee1 uniformly throughout the nucleus, and appears to increase the catalytic activity of both *Xenopus* and human Wee1 at least 5-fold [37, 38]. These two 14-3-3 dependent mechanisms of Wee1 activation work synergistically to enhance the phosphorylation and inactivation of nuclear Cdk1, and together with the 14-3-3-mediated nuclear exclusion and catalytic reduction of Cdc25B and C activity, acts as a 'belt and suspenders' mechanism to initiate a strong G2 checkpoint arrest (Figure 1A). Interference with either 14-3-3:Cdc25 or 14-3-3:Wee1 binding results in inappropriate mitotic entry [23, 24, 34, 38].

In contrast to the selectivity of Cdc25B/C for binding to 14-3-3 isoforms other than σ , it appears that Wee1 binds directly to 14-3-3 σ [38, 39], likely in addition to other 14-3-3 proteins. 14-3-3 σ , a unique isoform that appears to have first arisen in birds, appears to play an especially important role in G2/M checkpoint maintenance. Hermeking, Chan, Vogelstein and colleagues made the important finding that 14-3-3 σ was potently up-regulated by p53 in colorectal carcinoma cells after ionizing radiation, where it functioned in parallel with p21 to ensure that DNA damaged cells maintained a G2 arrested state [40–43]. Cells lacking 14-3-3 σ could initiate, but were unable to maintain a G2 arrest after irradiation [40], while cells lacking both 14-3-3 σ and p21 showed both accelerated cell death and enhanced sensitivity to doxorubicin-induced DNA damage and 5-fluorouracil treatment [41]. Possible targets of 14-3-3 σ may be Cyclin/Cdk1 and Cdk2 complexes themselves [40, 44], although neither of these targets was detected in an unbiased proteomic screen for 14-3-3 σ ligands [39]. Importantly, 14-3-3 σ likely functions, at least in part, through enhancing the activity of p53 as part of a coherent feed-forward loop ([45]; see below), as well as through enhancing the stability of the Cdk inhibitor protein p27Kip1 by blocking its AKT-mediated ubiquitination and degradation [46]. 14-3-3 regulation of p27Kip1 is more complex, however, since 14-3-3 bound p27Kip1 becomes re-localized in the cytoplasm, where it is presumably nonfunctional in suppressing Cdk activity [47]. This potentially allows tumor cells with high levels of PI 3-kinase/AKT activity to bypass G1 arrest (see below), while maintaining a sufficient total concentration of intracellular p27 to arrest the cell if DNA damage occurs subsequently.

14-3-3-binding sites on Cdc25, Wee-1, and a variety of other checkpoint modulators are generated through the action of DNA damage-responsive basophilic kinases, particularly the kinases Chk1, Chk2, and MK2 [22, 26, 48–52]. It is thought that Chk1 predominantly responds to single strand breaks, lesions created during replication fork collapse, and bulky

DNA adducts induced by UV irradiation, while Chk2 is thought to be the major kinase responding to double strand breaks, although these kinases appear to participate to varying extents in all three DNA damage responses [53]. Intriguingly, in fission yeast Chk1 itself binds to 14-3-3, and this binding appears to be important for phosphorylating and activating Chk1, and targeting it to the nucleus after DNA damage [54]. Recent data have now revealed that a third DNA damage kinase, the p38MAPK-activated kinase MK2, is critical for prolonged G2 arrest in tumor cells that are defective in p53 signaling [48, 49, 55]. The major direct targets of MK2 appear to be the Cdc25B and C phosphatases, which MK2 directly phosphorylates on their 14-3-3-binding motifs [48, 49, 56], and whose persistent 14-3-3-binding and nuclear exclusion requires persistent MK2 kinase activity. Importantly, the mechanism responsible for prolonged MK2 activity after DNA damage involves another example of a coherent feed-forward loop, in this case involving MK2-mediated phosphorylation of RNA binding proteins, which stabilize the production of the cell cycle regulator Gadd45 α , which in turn binds to p38MAPK to maintain prolonged MK2 activity [55]. The fact that this pathway is critical for preventing inappropriate checkpoint release and mitotic catastrophe in p53-defective cells suggests that it may be a useful therapeutic target to preferentially kill p53-defective tumor cells [49].

2.2 G2/M Checkpoint Release

Re-entry into the mitosis from G2 upon completion of DNA repair appears to involve Cdk1 and Plk1-dependent inactivation of the four upstream kinase pathways responsible for 14-3-3-dependent inactivation of CyclinB/Cdk1 itself - the ATM-Chk2 pathway, the ATR-Chk1 pathway, the p38MAPK-MK2 pathway (all of which target Cdc25 for binding to 14-3-3), and the Wee1 pathway (Figure 1B). Inactivation of the ATR-Chk1 pathway occurs through Plk1 mediated phosphorylation of Claspin, an adaptor protein that facilitates ATR-dependent Chk1 activation. Plk1 phosphorylation releases Claspin from damaged chromatin [57], and targets it for ubiquitin-mediated degradation [58–60]. Inactivation of the ATM-Chk2 pathway involves the Cdk1 and Plk1 dependent inactivation of the adaptor protein 53BP1 (that facilitates sustained Chk2 activation by ATM as well as direct inactivation of Chk2 itself in both mammalian cells and yeast [61, 62]. Inactivation of the p38MAPK-MK2 pathway is less well understood, but likely involves the dephosphorylation and inactivation of p38MAPK by the phosphatase Wip1 [63, 64], which also dephosphorylates the DNA damage marker histone γ H2AX along with other ATM/ATR and DNA-PK targets [65, 66]. Inactivation of the Wee1 pathway involves the Cdk1 and Plk1 phosphorylation of Wee1, which targets it for ubiquitin-mediated proteolysis [67, 68]. These mechanisms function together to block upstream signals that target Cdc25B/C and Wee1 for 14-3-3 binding, thus shutting off the G2/M checkpoint regulated by 14-3-3-mediated Cdc25 inactivation and Wee1 activation, and allowing cells to re-enter the cell cycle.

The process of checkpoint release is still under active investigation. This is particularly important since it appears that G2/M checkpoint bypass may be an early event that triggers the progression from carcinoma in situ to full blown cancer [69]. In addition, the G2/M checkpoint appears to be an error-prone checkpoint, since both normal cells and tumor cells are released from G2 into M-phase despite the presence of a finite number of unrepaired DNA breaks [70], resulting in genomic instability. Taken together, these findings suggest that modulation of 14-3-3 function to enhance G2 arrest may be a viable mechanism to limit tumor cell proliferation and progression.

An important observation made by Kornbluth and colleagues was that 14-3-3 binding to *Xenopus* Cdc25 phosphorylated on S287 protected this mitotic phosphatase from premature dephosphorylation and activation [71]. In addition, protein phosphatase 1 was required to dephosphorylate *Xenopus* Cdc25 at S287 (the 14-3-3 binding site corresponding to S216 in human Cdc25C) [72]. Interestingly, the removal of 14-3-3 from Cdc25 preceded S287

dephosphorylation, implying that a phosphatase-independent pathway is responsible for the initial 14-3-3 removal from Cdc25, followed only later by dephosphorylation and loss of the 14-3-3 binding site on Cdc25. These authors postulated that the accumulation of phosphorylated intermediate filament proteins during mitosis may compete with Cdc25C for 14-3-3 binding, with a shift in the equilibrium away from 14-3-3:Cdc25C as the abundance of phosphorylated forms of intermediate filament proteins accumulate in mitosis. These findings agree well with a prior report from Tzivion et al. [73] that treatment of COS-7 cells with the phosphatase inhibitor Calyculin-A resulted in marked accumulation of 14-3-3:phosphovimentin complexes, displacing many of the other 14-3-3-bound ligands, and a series of strong papers from Omary and colleagues that showed binding of phosphorylated epithelial keratins K8/18 to 14-3-3 is increased during mitosis, where it may contribute to mitotic progression, at least in hepatocytes during liver regeneration [74, 75]. These studies raise the possibility that intermediate filament phosphorylation during mitosis may function to facilitate the release of specific ligands from 14-3-3 whose function is important during cell division by competitive binding, although additional mechanisms are likely to be operative during the initial stages of checkpoint release when mitotic kinase activity may not be high enough to produce an abundance of phosphokeratins and phosphovimentin. Importantly, the 14-3-3 strongest binding motif on Cdc25B and C corresponds to the sequence RSP**S**MP, where the bolded highlighted Ser is the critical 14-3-3-binding phosphorylation site. Once cells have entered mitosis, Cdk activity results in phosphorylation of the preceding Ser residue (RSP**S**MP), which renders these Cdc25 molecules incapable of binding to 14-3-3 proteins [76, 77], and thereby ensuring that mitotic progression is strictly unidirectional.

The fact that Cdk1 is intimately involved in nearly all of the G2 checkpoint release mechanisms discussed above shows that, like checkpoint establishment, checkpoint release also involves a feed-forward loop when viewed from a systems biology perspective.

2.3 Mitotic roles of 14-3-3 proteins

In addition to from blocking mitotic entry, 14-3-3 proteins appear to contribute substantially to enhancing progression once cells have entered mitosis through stimulating the process of cytokinesis. Parker and colleagues showed that 14-3-3 binding to protein kinase C epsilon (PKC ϵ) during mitosis activates it in a lipid-independent manner, locking PKC ϵ in an open active conformation near the actomyosin ring. The resulting PKC ϵ activity is crucial during telophase to down-regulate RhoA activity at the midbody, and allow cells to complete abscission. Loss of the 14-3-3 binding sites on PKC ϵ , loss of PKC ϵ expression, expression of a catalytically dead form of PKC ϵ or expression of a dominant negative form of 14-3-3 results in cytokinesis delay or failure and the production of multinucleated cells [78]. The cancer relevance of this phenomenon is unclear, since PKC ϵ is generally thought to be a potent oncogene and its expression is upregulated in certain tumors and tumor-derived cell lines [79].

Our laboratory showed that 14-3-3 σ plays an important role in cytokinesis by facilitating the mitotic switch from cap-dependent to cap-independent translation. Notably, 14-3-3 σ is a tumor suppressor protein that is frequently lost in tumors of epithelial origin [80], inhibiting the ability of such cells to efficiently complete cytokinesis. This ultimately leads to the production of tetraploid cells that may serve as early precursors of cancer [81]. In normal epithelial cells, completion of normal cytokinesis occurs as a consequence of binding of the translation initiation factor eIF4B to 14-3-3 σ during mitosis, impairing eIF4B function, and culminating in a switch from cap-dependent to cap-independent (IRES mediated) mRNA translation during mitosis. During mitosis, a variant form of Cdk11, termed p58-PITSLRE, is then translated in a cap-independent manner [82]. This cap-independent form is

specifically required to complete the final stages of cell division [83, 84]. In the absence of 14-3-3 σ , only the cap-dependent interphase form of Cdk11, p110-PITSLRE is produced, rendering 14-3-3 σ -defective cells unable to sever the cell-cell attachment at the terminal midbody stage (abscission) of cell division [81]. These findings are particularly significant given the finding that the expression of 14-3-3 σ is suppressed in normal breast tissue adjacent to the sites tumors arose (see below). In this case, loss of 14-3-3 probably contributes to 'field cancerization' by facilitating the development of pre-cancerous tetraploid lesions that subsequently undergo genomic instability during the tumorigenic process.

2.4 G1/S Checkpoint control

The remaining Cdc25 phosphatase that has not been discussed at length, Cdc25A, is best appreciated for its role in regulating the G1/S transition by controlling CyclinE and CyclinA/Cdk2 activity. Cdc25A has been generally accepted as being primarily regulated in response to DNA damage by Chk1-dependent phosphorylation and β -TrCP-mediated ubiquitination and proteosomal degradation [85–87]. Other kinases however, including CK1, GSK-3 β , and Plk3 have also been implicated in this process [88–90]. In addition to its role in the G1/S transition, Cdc25A also appears to play a role in facilitating the G2/M transition by activating CyclinB/Cdk1 [91]. Piwnicka-Worms and colleagues showed that Cdc25A has two 14-3-3-binding sites, and that mutants of Cdc25A that are defective in 14-3-3 binding have an enhanced ability to activate Cyclin B/Cdk1 [92]. One of the two 14-3-3-binding sites sits near the C-terminal region of Cdc25A, immediately adjacent to a putative Cyclin B docking site, suggesting that 14-3-3 binding disrupts the interaction of Cdc25A with its substrate. These data suggest that 14-3-3-binding to Cdc25A may be important for short-term control of Cyclin/Cdk complexes involved in both G1/S and G2/M progression, while ubiquitin-mediated destruction of Cdc25A may be a mechanism of long term inhibition of Cyclin/Cdk activation in response to larger amounts of genotoxic stress. Unfortunately, the structural basis for 14-3-3-mediated effects on Cdc25 phosphatases remains unclear, largely because the 14-3-3-binding sites are primarily localized in relatively unstructured regions of Cdc25 between the N-terminal regulatory domain and the C-terminal phosphates domain.

An additional role for 14-3-3 proteins in the G1/S transition has emerged from a recent finding that 14-3-3 τ seems to facilitate the MDM2-dependent but ubiquitin-independent degradation of the Cdk inhibitor p21 to facilitate S-phase entry [93] (Figure 1B). Thus 14-3-3 proteins appear to have roles in both suppressing and enhancing the G1/S transition, likely as a function of which client proteins are bound, depending on the relative levels of activity of different 14-3-3 motif-generating kinases.

3. 14-3-3 proteins and apoptosis

3.1 14-3-3 binding to Bcl-2 family members

An important role for 14-3-3 proteins in apoptosis emerged from a series of studies examining the interaction of 14-3-3 proteins with BH3 domain-containing proteins, particularly BAD and BAX. Early work identified BAD as a BH3 domain-only protein that was the 14-3-3 binding target of an IL-3 dependent survival mechanism in the hematopoietic cell line FL5.12 [94]. BAD binding to 14-3-3 proteins was found to involve the AKT-dependent phosphorylation of two sites on BAD [95], although additional kinases also may be involved [96]. The mechanism by which 14-3-3 facilitates the inactivation of BAD involves both transient sequestration of BAD to prevent it from binding to and inactivating the pro-survival protein Bcl-XL, along with holding BAD in a conformation that enhances the phosphorylation of an additional residue in its BH3 domain to result in a more permanent state of inactivation [97].

14-3-3 proteins were later shown to bind to, and functionally inactivate the critical pro-apoptotic effector BAX, in a phospho-independent manner [98]. The binding involves sequences at both the N- and C-terminus of BAX, and precludes BAX from transiting to the mitochondria, where it would otherwise interact with BAK to induce permeabilization of the mitochondrial outer membrane and trigger the release of cytochrome C and SMAC/DIABLO to activate caspases to kill the cell [99]. More recent work has now shown that this 14-3-3:BAX interaction is controlled by the stress MAP kinase JNK, which directly phosphorylates 14-3-3 on Ser-184 (14-3-3 ζ notation) near the end of helix α 7 to block BAX binding [100].

3.2 14-3-3-mediated control of signaling components and transcription factors involved in the apoptotic response

JNK, which triggers the release of BAX from 14-3-3, as well as p38MAPK, are activated, in part by the upstream MAP kinase kinase kinase ASK1 (apoptosis stimulating kinase 1) [101], which is itself negatively regulated by 14-3-3 binding [102]. ASK1 is activated downstream of the TNF α receptor and FAS. TNF α receptor-mediated apoptosis is negatively regulated by the zinc finger protein A20 [103], and just like ASK1, A20 is also a client protein whose function is repressed by binding to 14-3-3 [104]. Signaling from TNF receptor family members to BAX involves formation of a complex between the receptors and the proteins MOAP-1 and RASSF1A, a process that is inhibited by 14-3-3 binding to RASSF1A [105, 106]. Finally, in response to growth factor deprivation, and potentially other pro-apoptotic stimuli, members of the FOXO family of transcription factors drive the expression of a pro-apoptotic gene expression program [107]. Not surprisingly, then, a number of FOXO transcription factors have been shown to be under the control of 14-3-3 proteins, where their binding is regulated by AKT-dependent phosphorylation [108–111]. Intriguingly, both AKT-dependent phosphorylation of FOXO proteins and 14-3-3 binding appears to occur in the nucleus [110], resulting in both an inability of FOXO proteins to bind to DNA [112–114] and facilitating their export out of the nucleus through a combination of masking NLS sequences and exposing NES sequences [110]. Thus, 14-3-3 proteins control the induction of apoptosis at multiple levels, from effector BH3 domain-containing proteins to upstream activators and transcription factors. In good agreement with this, Masters and Fu showed that blocking of the 14-3-3 binding cleft with a peptide inhibitor (see below) enhanced the apoptotic response of multiple cell lines in culture [115] while Muslin and colleagues showed that transgenic expression of a dominant negative form of 14-3-3 could increase the apoptotic response of cardiomyocytes in the hearts of mice to cardiac pressure overload. The observation that direct phosphorylation of 14-3-3 by JNK disrupted binding of 14-3-3 to BAX and Forkhead transcription factors (and likely other 14-3-3 targets such as A20 and ASK1) [116], again illustrates the importance of feedforward and feedback loops in 14-3-3 mediated control of cell fate, since once a threshold of JNK activity has been exceeded, one would expect additional release of ASK1 and RASSF1A to enhance further JNK activity, BAX release and activation, and transcription of FOXO-driven apoptotic genes, while release of A20 would limit subsequent upstream input into this pathway from the TNF receptor, and allow additional pro- and anti-apoptotic pathways to be integrated into the overall response.

3.3 Therapeutic targeting 14-3-3: ligand binding by drugs

The idea that global interference of 14-3-3 proteins with their target ligands could be used therapeutically was first explored by Fu and colleagues through the use of a non-phosphorylated 20 amino acid binding peptide, R18, that was isolated from a phage display library by virtue of its strong binding to 14-3-3 [117]. Difoepin, a high affinity 14-3-3 antagonist, was then constructed by linking two R18 peptides together with a linker, thereby blocking both binding sites within a functional 14-3-3 dimer. Treatment of several cancer

cell lines with Difopein resulted in enhanced apoptosis both alone, and when administered in combination with cisplatin [115], although the death was additive and not synergistic. An alternative approach taken by our laboratory involved the use of photo-activatable caged phosphopeptides to allow timed inhibition of 14-3-3 proteins, and this was shown to enhance UV-induced cell death [118]. Such an approach might have utility in various form of photodynamic therapy, such as that used for treatment of mycosis fungoides. However, a general caveat of both of these approaches is their non-isoform specificity, and the potential for significant toxicity in normal non-cancerous tissue. The recent surge of interest in RNAi-mediated therapeutics might be an ideal strategy for targeting specific members of protein families, such as particular 14-3-3 isoforms whose up-regulation may directly contribute to the oncogenic behavior of tumor cells and their resistance to anti-cancer agents.

Recently, the first non-peptidic 14-3-3 small molecule inhibitor was reported [119], and shown to enhance the apoptotic cell death of chronic myelogenous leukemia cells [120]. An alternative approach to killing cancer cells might be to block their proliferation by stabilizing the interaction of cell cycle regulators such as Wee1 and Cdc25 with 14-3-3 proteins. Interestingly, two small plant toxins, fusicoccin A and cotylenin A, bind to 14-3-3 and stabilize the interaction between 14-3-3 proteins and a subset of client proteins [121, 122], raising the possibility that this approach might be adapted for use in mammalian cells [123].

4. Conclusions

By functioning as master regulators of protein kinase signaling cascades and effector proteins involved in cell cycle control and apoptosis, 14-3-3 proteins function as signal transduction ‘integrators’ that connect signals to phenotypes. The diversity of 14-3-3 isoforms and their interactions reveals an abundance of potential drug targets that could be used to therapeutically treat diseases caused by aberrant cell proliferation such as cancer, as well as those diseases resulting from excessive inflammation and cell death, including degenerative diseases associated with aging. Examination of the signaling pathways in which 14-3-3 proteins participate reveals the striking importance of feedforward and feedback loops in the fine control of 14-3-3 regulated signaling networks.

Highlights

1. Checkpoint kinases use 14-3-3 proteins to establish and maintain a G2 cell cycle checkpoint by targeting Cdc25 and Wee1.
2. Cdk1 and Plk1 inactivate checkpoint kinase pathways targeting Cdc25B and Wee1 binding to 14-3-3 to drive mitotic entry.
3. 14-3-3 proteins prevent apoptosis by binding to BH3-containing proteins, FOXO transcription factors and apoptotic kinases.
4. JNK directly phosphorylates 14-3-3 proteins to block their anti-apoptotic effects and drive cell death.

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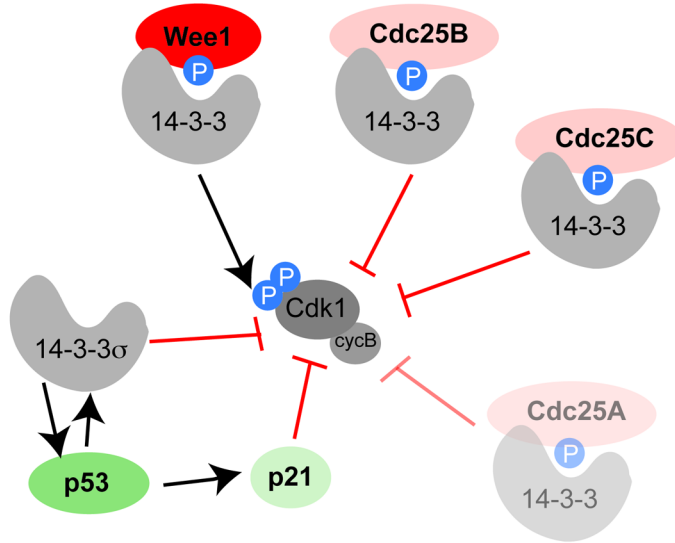
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A G2/M Checkpoint Initiation and Maintenance



B G2/M Checkpoint Recovery

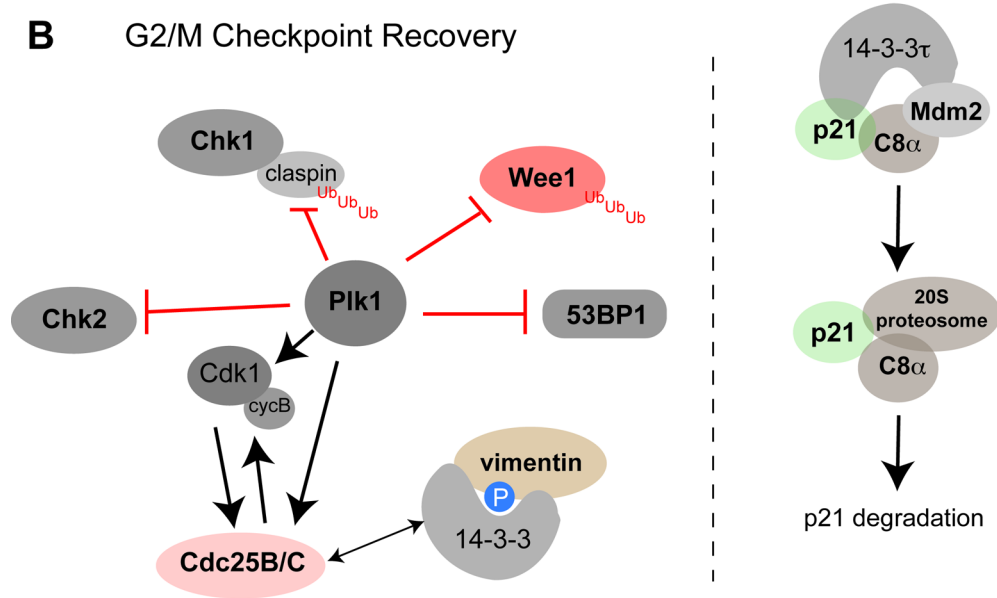


Figure 1. The role of 14-3-3 in G2/M checkpoint activation, maintenance, and release
(A) 14-3-3 proteins inhibit cyclinB-Cdk1 activators and activate Cdk1/cyclin B inhibitors. See text for details. **(B)** Mitotic reentry following recovery from the G2 checkpoint involves dissociation of the 14-3-3 proteins from the ligands shown in panel A and inactivation of the upstream kinase pathways responsible for their 14-3-3 binding. Dynamic balance between 14-3-3 mediated Cdk1/cyclin B inhibition and Cdk1/Plk1-mediated recovery maintains cells in a state of temporary arrest that is likely to be defective in tumor cells. In addition, a role for 14-3-3 in p21 degradation has been shown to be involved in G1/S progression and could

also be important in M phase entry from G2, rationalizing the observation that 14-3-3 proteins are overexpressed in certain tumor types.

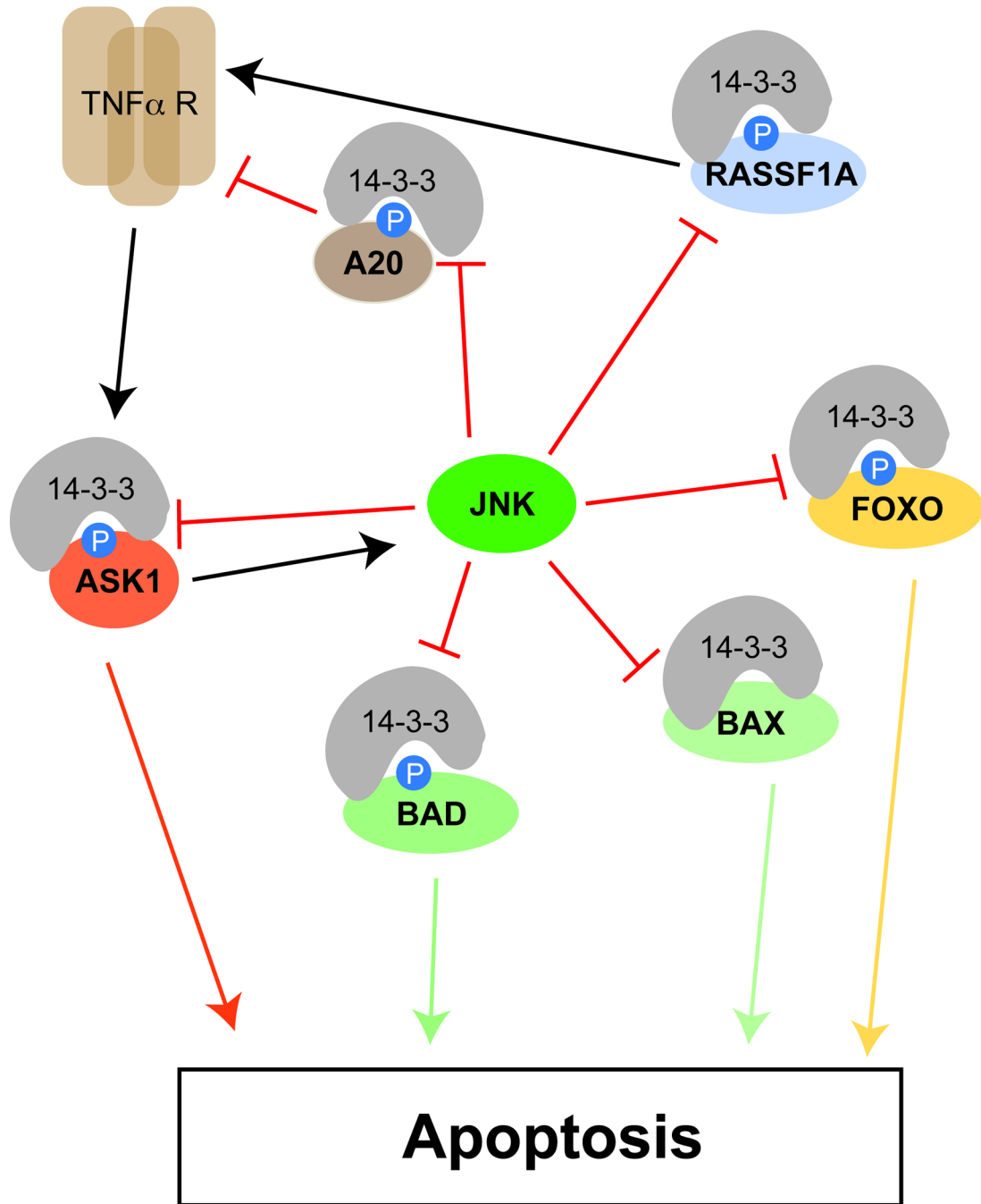


Figure 2. Roles for 14-3-3 proteins at multiple points in the apoptotic signaling network
 14-3-3 proteins bind numerous effectors of apoptosis, shaded orange, yellow and green, to inhibit their pro-apoptotic function (see text for details). Apoptotic activation is facilitated by JNK-mediated phosphorylation of 14-3-3, triggering release of these apoptotic effectors. This can occur, for example, through a feedforward loop activated through the TNF- α receptor.