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Review

Cyclin-dependent kinase-1: linking apoptosis to cell cycle and mitotic catastrophe

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Abstract

The cyclin-dependent kinase 1 (Cdk1), formerly called Cdc2 (or p34^{Cdc2}), interacts with cyclin B1 to form an active heterodimer. The activity of Cdk1 is subjected to a complex spatiotemporary regulation, required to guarantee its scheduled contribution to the mitotic prophase and metaphase. Moreover, the activation of Cdk1 may be required for apoptosis induction in some particular pathways of cell killing. This applies to several clinically important settings, for instance to paclitaxel-induced killing of breast cancer cells, in which the ErbB2 receptor kinase can mediate apoptosis inhibition through inactivation of Cdk1. The activation of Cdk1 participates also in HIV-1-induced apoptosis, upstream of the p53-dependent mitochondrial permeabilization step. An unscheduled Cdk1 activation may contribute to neuronal apoptosis occurring in neurodegenerative diseases. Finally, the premature activation of Cdk1 can lead to mitotic catastrophe, for instance after irradiation-induced DNA damage. Thus, a cell type-specific modulation of Cdk1 might be taken advantage of for the therapeutic correction of pathogenic imbalances in apoptosis control.
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Abbreviations: AIF, apoptosis inducing factor; FAD, flavine adenine dinucleotide; NAD, nicotine amide dinucleotide

The cyclin-dependent kinase 1 (Cdk1), formerly called Cdc2 (or p34^{Cdc2}), interacts with cyclin B1 to form an active heterodimer, the 'mitosis-promoting factor' whose activity determines the cell cycle timing of mitosis. In addition, Cdk1 activation has initially been suggested to be a stringent requirement for the entry into apoptosis.[1] However, several studies have shown that apoptosis can occur in postmitotic neurons in the absence of detectable Cdk1 expression,[2] and in both serum-deprived fibroblasts and immature thymocytes, apoptosis can proceed without activation of the low level of Cdk1 found in these cells.[3,4] Although, this eliminates the possibility that Cdk1 would be part of the central executioner of apoptosis, recent data indicate that Cdk1 does play an important role in some particular lethal signal transduction pathways, as will be discussed in this review.

Regulation of the cyclin B/Cdk1 complex during mitosis

Progression from G2- to M-phase is driven by activation of the Cdk1/cyclin B1 complex, whose activity must be sustained from the prophase to the metaphase, within the nucleus. Subsequent entry into the anaphase critically relies on the sudden destruction of the Cdk1/cyclin B activity.[5,6] Accordingly, the activity of Cdk1 is regulated in a spatiotemporary pattern at several levels, namely (1) at the level of transcription of cyclin B1 and (to a lower extent on that of Cdk1); (2) at the level of regulatory Cdk1 phosphorylations; (3) at the level of the subcellular distribution of cyclin B1; and (4) at the level of the regulated degradation of cyclin B.

The transcription of Cdk1 is rather stable throughout the cell cycle, although it is modulated in pathological conditions. For instance, p53 negatively regulates Cdk1 transcription[7] as well as that of cyclin B1.[8] The cyclin B gene is transcribed and its mRNA is stabilized from the end of the S-phase. The phosphorylation of Cdk1 on Thr^{14} (mainly by Myt1 kinase) and Tyr^{15} (mainly by Wee1 kinase) inhibits its activity during the G_2-phase of the cell cycle, presumably through a direct effect on the phosphotransfer activity, while de-phosphorylation of Cdk1 on Thr^{14} and Tyr^{15} by the phosphatase Cdc25C de-inhibits Cdk1 during early mitosis. The regulation of Cdc25 phosphatase activity by Cdk1 itself, as well as by other phosphatases and kinases, is complex (Figure 1). The phosphorylation of Thr^{161} by CAC (Cdk activating kinase, a heterodimer of cyclin H and Cdk7), is strictly required for Cdk1 to be active. The Cdk1 inhibitor p21$^{Cip1/Waf1}$ directly inhibits Cdk1 activity. Cyclin B1, the obligate allosteric activator of Cdk1, changes its subcellular localization from the cytosol to the nucleus, early during mitosis. The equilibrium between nuclear import and export of cyclin B1 (as well as that of Cdc25C, required for activation of Cdk1) is influenced by its phosphorylation status.[5,6] At the end of the metaphase, a specialized device, the anaphase promoting complex (APC) must destroy cyclin B to allow mitosis to proceed. The APC, a polyprotein complex activated by Cdc20 recruits cyclin B, causes its ubiquitination, and thus targets it for degradation by the 26S proteasome.[9]

The Cdk1/cyclin B heterodimer induces mitosis by phosphorylating and activating enzymes regulating chro-

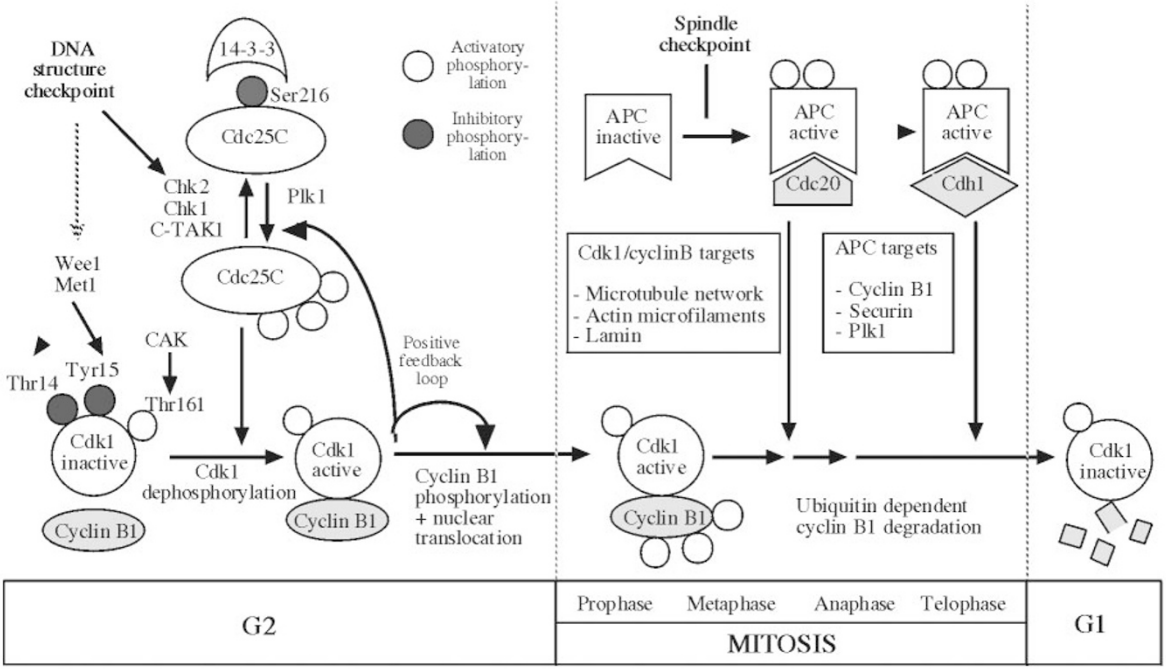

Figure 1 Regulation of Cdk1 during normal mitosis. To allow cells to progress from the G2 to the M phase, Cdk1 has to be active, meaning that it has to bear an activating phosphorylation on Thr^{161} by CAK, that the inhibitory phosphorylation (on Thr^{14} and Tyr^{15}) has been removed by active Cdc25C phosphatase, and that Cdk1 associates with cyclin B and translocates to the nucleus. In the nucleus, the active Cdk1/Cyclin B complex then phosphorylates mitotic substrate proteins. During the anaphase the APC becomes activated and targets Cdk1 for degradation. Note that two cell cycle checkpoints indirectly affect the activity of Cdk1. The DNA structure checkpoint activates checkpoint kinases (such as Chk1 and Chk2), which phosphorylate Cdc25C on Ser^{216}, thereby causing it inactivation (and failure to activate Cdk1). Activation of the spindle checkpoint delays maturation of the APC (and thus prevents cyclin B degradation)

matin condensation, nuclear membrane breakdown, mitosis-specific microtubule reorganization, and the actin cytoskeletion allowing for mitotic rounding up of the cell.[5,6] It is important to note that the mitotic and apoptosis rounding up of the cell and dissolution of the nuclear envelope are fundamentally different. Thus, the mitotic dismantling of the envelope is catalyzed by kinases (and in part by Cdk1/cyclin B) which phosphorylate lamins, thereby causing their reversible depolymerization. In contrast, apoptosis involves the irreversible proteolytic cleavage of lamin B by caspase-6.[3,10]

The perfect regulation of the spatiotemporal pattern of Cdk1/cyclin B activity is pivotal for the normal cell cycle and is subject to multiple control steps. Thus, the so-called 'DNA structure checkpoints' (which are activated by incomplete DNA replication or by DNA damage such as strand breaks) will stimulate Wee1/Myt1 as well as so called check point kinases (Chk1, Chk2, which inactivate Cdc25C) to prevent Cdk1 activation and entry into mitosis. Similarly, the APC can be inhibited during the 'spindle assembly checkpoint', thus preventing the degradation of Cdk1 (and that of many other substrates) and nuclear division[5,6,11] (Figure 1).

Cdk1 as a target for apoptosis induction?

Most if not all perturbations of cellular physiology can lead to apoptosis as a default pathway,[12] and this apparently also applies to the induction of cell death by inhibition of Cdk1. Using a temperature sensitive Cdk1 mutant cell line, it was shown that inactivation of Cdk1 increases the level of apoptosis induced by the topoisomerase II inhibitors mitoxantrone and teniposide.[13] Similarly, down-regulation of Cdk1 in a cell line in which endogenous Cdk1 gene expression depends on the presence of an inducer in the culture medium, results in extensive DNA replication and apoptosis.[14] Nonethless, there are little data suggesting that pharmacological inhibition of Cdk1 will cause apoptosis in addition to arresting the cell cycle during the G2 phase. Flavopiridol, an inhibitor of Cdk1 (and other cyclin dependent kinases) causes apoptosis in a variety of different tumor cells, including in pre-clinical mouse models[15] and in clinical trials.[16] Nevertheless, it appears probable that flavopiridol induces apoptosis by curtailing the activity of other kinases than Cdk1, for instance that of Cdk9, which is required for transcription.[16]

How can inhibition of Cdk1 lead to apoptosis? One interesting Cdk1 target is survivin, which is phosphorylated on Thr^{34}.[17] Loss of phosphorylation on this residue, as a result of a genetic manipulation of survivin, can result in the dissociation of a survivin-caspase-9 complex normally anchored on the mitotic midbody, thereby causing caspase-9-mediated apoptosis of cells traversing mitosis.[17] It has also been speculated that Cdk-1 mediated phosphorylation of Bcl-2 might be cytoprotective, at least in a model of hypericin-mediated photodynamic therapy.[18] Whether the absence of phosphorylation of survivin and/or Bcl-2 truly explains the pro-apoptotic effect of Cdk1 inhibition, however, remains an ongoing conundrum, and it is formally

possible that phosphorylation of Bcl-2 constitutes a marker of mitosis, without any major relevance to the regulation of apoptosis.

Cdk1 as a pro-apoptotic mediator

An increase in Cdk1 activity has been found in numerous apoptotic conditions, and inhibition of the Cdk1/cyclin B complex by a dominant-negative (DN) Cdk1 mutant, antisense constructs, or chemical inhibitors have suggested that this increase in activity may be indeed important for cell killing (Table 1). In Jurkat cells, crosslinking of CD95/Fas/Apo1 causes a rapid activation of Cdk1 due to the caspase-3 mediated cleavage of Wee1 (and consequent de-phosphorylation of Cdk1 on Tyr^{15}),[19] suggesting that the activation of Cdk1 observed in cell death in some cases is a consequence rather than a mechanism of apoptosis. It may also be criticized that Cdk1 inhibition will cause a G2 arrest and thus prevent apoptosis in a indirect fashion related to the cell cycle arrest. Nonetheless, several authors have suggested that Cdk1 can act as a pro-apoptotic mediator, as will be discussed below.

From a clinical point of view, perhaps the most interesting observation concerns Cdk1 activation by microtubule inhibitors such as paclitaxel (Taxol). Taxol has been found to increase the activity of Cdk1 in several breast cancer cell lines, presumably as an indirect consequence of its effects on microtubuli and the cell cycle. Chemical inhibition of Cdk1 or transfection with DN-Cdk1 blocks taxol-induced cell death[20,21] (Figure 2). In this system, 12-O-eteradecanaoylphorbol-13-acetate (TPA), a protein kinase C activator and tumor promoter, can prevent the taxol-mediated Cdk1 activation without affecting the taxol-induced tubulin polymerization.[21] Moreover, transfection-enforced overexpression of $p185^{ErbB2}$ protein, a plasma membrane-anchored receptor tyrosine kinase, blocks Cdk1 activation by at least two different mechanisms. First, ErbB2 causes the upregulation of $p21^{Waf1/Cip1}$, which interacts with and inhibits Cdk1.[21] The absence of p21 also sensitizes HCT116 colon carcinoma cells to the induction of apoptosis by taxol and vincristine, correlating with a prolonged Cdk1 activity and the occurrence of endoreduplication.[22] As a second mechanism of Cdk1 inhibition, ErbB2 can directly phosphorylate Cdk1 on Tyr^{15} (the site also targeted by Wee1); thereby causing its inactivation.[23] This is a clinically important pathway of taxol resistance because ErbB2-positive mammary cancers are relatively resistant to taxol. As shown in a phase II clinical trial, such cancers become responsive to taxol *in vivo* when taxol is combined with Herceptin (Trastuzumab), a humanized monoclonal anti-ErbB2 antibody.[24]

In HL60 myelomonocytic leukemia cells, the taxol-induced Cdk1 activation is not affected by Bcl-2 nor is it inhibited by the pan-caspase inhibitor Z-VAD.fmk, although Bcl-2 (but not Z-VAD.fmk) does prevent the mitochondrial release of cytochrome *c*.[25] This places Cdk1 upstream of the Bcl-2-regulated mitochondrial changes that define apoptosis.[12,26] Intriguingly, two observations suggest that Cdk1 can induce mitochondrial membrane permeabilization by acting on proteins from the Bcl-2 family. First, Cdk1 can phosphorylate the pro-apoptotic Bcl-2 protein family member Bad on Ser^{128}, thereby causing a loss of interaction between Bad and cytosolic 14-3-3 proteins.[27] As a result, Bad can translocate to mitochondria, where it antagonizes with Bcl-2-like proteins and/or activates Bax-like proteins[28] and causes mitochondrial membrane permeabilization and consequent cell death (Figure 2). Thus, at difference with other anti-apoptotic Bad kinases which phosphorylate Ser^{136} (Akt/PKB, PAK1, $p70^{S6k}$) or Ser^{112} (Rsk, PKA, PAK1), thus favoring the interaction between Bad and 14-3-3, Cdk1 is a pro-apoptotic Bad kinase. Indeed, the Ser^{128} phosphorylation of Bad inhibits the interaction of Ser^{136}-phosphorylated Bad with 14-3-3 proteins, indicating that Cdk1 can interfere with survival signaling mediated by Akt/PKB, PAK1 and $p70^{S6k}$.[27] In addition, Cdk1 may phosphorylate and inactivate Bcl-2 on Ser70 and Ser87, that is within a proline-rich loop associated with autorepression of its anti-apoptotic activity.[29] However, several groups failed to demonstrate direct Bcl-2 phosphorylation by Cdk1 *in vitro*,[18,30] and the functional consequences of the Bcl-2 Ser^{70} phosphorylation are controversial.[18,31]

Unscheduled expression and activation of Cdk1/cyclin B has been seen in neurons undergoing degeneration in Alzheimer's disease, as well as in other neurodegenerative conditions, correlating with accumulation of mitotic phosphoepitopes.[32] This change may be attributed to an

Table 1 Models of apoptosis induction involving Cdk1

Model	Observation	Reference
HeLa cells treated with staurosporine or TNF-α	DN Cdk1 mutant inhibits apoptosis	63
Crosslinking of CD95 in Jurkat cells	Activation of Cdk1 activity DN-Cdk1 or Wee 1 inhibit apoptosis	64
γ-Irradiation of hematopoietic cells	Accumulation of cyclin B1 Cyclin B1 anti-sense oligo inhibits death	65
Activation of T cell hybridoma	Increased kinase activity of Cdk1 on H1 Cyclin B anti-sense oligonucleotide inhibits cell death	66
Taxol on MCF-7 breast cancer cells	Increased Cdk1 activity Oloumycin (which inhibits Cdk1) inhibits apoptosis	20
Taxol on MDA-MB-435 breast cancer cells	Increased Cdk1 activity TPA inhibits Cdk1 and apoptosis	21
Cerebellar granule neurons subjected to KC1 deprivation	Increased Cdk1 expression and activity DN-Cdk1 inhibits apoptosis	27
HIV-1-Env/CD4 mediated fusion of HeLa cells	Increased cyclin B and Cdk1 activity DN-Cdk1 inhibits apoptosis	49
HIV-1 infection of $CD4^+$ lymphoblasts	Oloumycin and roscovitine inhibit apoptosis	49

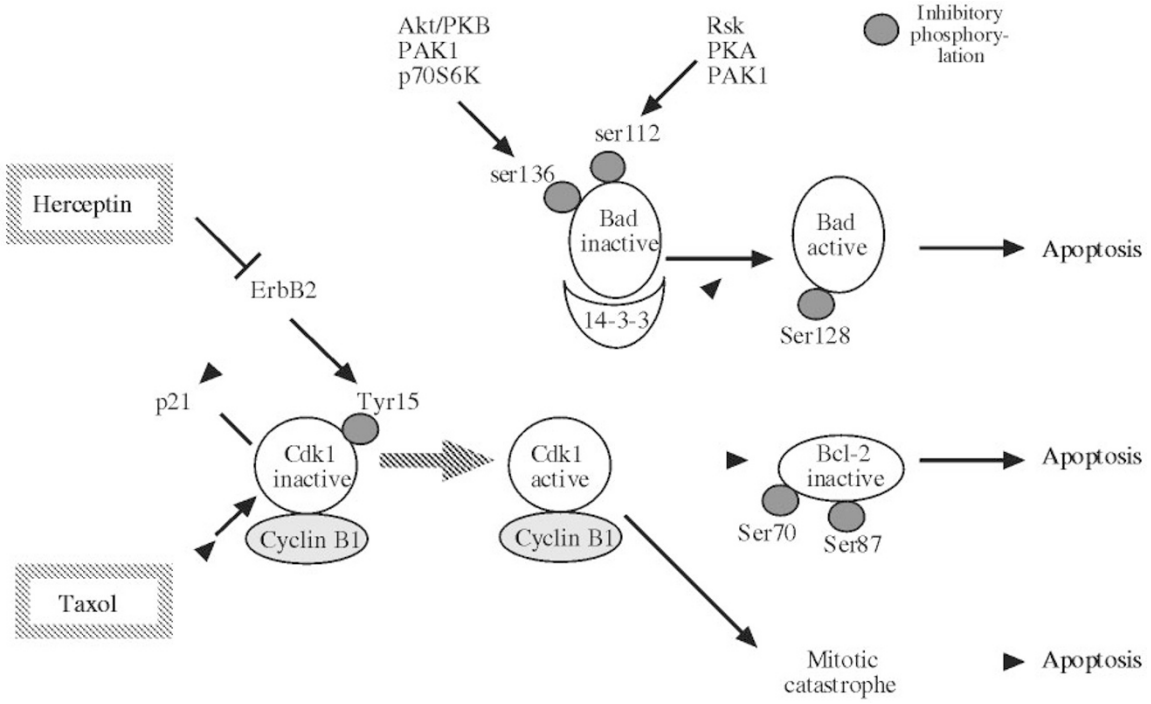

Figure 2 Involvement of Cdk1 in apoptosis. In breast cancer cells expressing ErbB2, the simultaneous application of Taxol (paclitaxel, which indirectly activates Cdk1) and Herceptin (a monoclonal ErbB2-inhibitory antibody) synergize to induce apoptosis, presumably because Herceptin relieves an ErbB2-mediated block of Cdk1. Cdk1 can induce cell death by triggering premature entry into mitosis and consequent mitotic catastrophe, followed by apoptosis. In addition, Cdk1 can phosphorylate the pro-apoptotic protein Bad, causes its removal from its site of action, the mitochondrion. Alternatively, Cdk1 can phosphorylate (and inactivate?) the anti-apoptotic protein Bcl-2

increase in Cdc25B tyrosine phosphatase activity.[33] However, thus far no evidence has been published, to our knowledge, that inhibition of Cdk1/cyclin B can protect against neurodegeneration.

Cdk1 in mitotic catastrophe

Mitotic catastrophe is the form of cell death that results from aberrant mitosis, leading to the formation of large non-viable cells with several micronuclei containing uncondensed chromosomes. Although apoptosis may be a consequence of mitotic catastrophe, it would be a misconception to assume that apoptosis would be required for the lethal effect of mitotic catastrophe.[34] Although the expression of anti-apoptotic proteins such as Bcl-2 or MDA can prevent apoptosis occurring after mitotic catastrophe, it does not improve clonogenic survival.[34,35] Mitotic catastrophe can result from deficient mitotic checkpoints in tumor cells, anti-microtubular drugs, and premature mitosis. All these conditions may involve the unscheduled activation of Cdk1. For instance, the fusion of mitotic cells with interphase cells in S or G2 can result in mitotic catastrophe, presumably due to the premature induction of mitosis before the completion of S or G2.[36] This premature induction obviously involves the 'mitosis-promoting factor', Cdk1/cyclin B1. Accordingly, ectopic overexpression of cyclin B plus Cdk1 can result in premature chromatin condensation and mitotic catastrophe.[37,38] Similarly, in cells expressing p21 under the control of an inducible promoter, the sudden reduction of p21 expression (which de-represses Cdk1) can lead to mitotic catastrophe after prolonged growth arrest.[39]

Mitotic catastrophe may be a clinically important modality of cell death, because DNA damage, for instance induced by γ-irradiation, which normally activates the DNA damage checkpoint (Figure 1, 2), fails to do so in cancer cells lacking sensors of DNA damage (e.g. ATM, ATR), adaptors (e.g. BRCA1) or effector kinases (e.g. Chk1, Chk2) required for cell cycle arrest.[11] This may explain why cancer cells are particularly sensitive to the induction of mitotic catastrophe. Moreover, chemical inhibitors of check point kinases can sensitize tumor cells to the induction of mitotic catastrophe (and subsequent apoptosis).[40–43]

Cdk1 in the cytopathogenicity of human immunodeficiency virus-1 (HIV-1)

Peripheral CD4 and CD8 cells from HIV-1-infected donors overexpress cyclin B and frequently contain two nucleolar organizer regions (NOR), which is a marker of the G2 phase. These alterations return to normal after successful anti-retroviral therapy.[44,45] The increased expression of cyclin B has been correlated with an enhanced half-life of the cyclin B protein and is associated with a decreased level of cyclin B ubiquitination.[45]

In vitro, $CD4^+$ T cells exposed to cells expressing the HIV-1 envelope (Env) gene can undergo cell death while arresting in the G2/M-phase without any significant formation of syncytia. Such cells manifest the inhibitory

hyperphosphorylation of Cdk1, as well as an increase in cyclin B.[46] Coculture of HeLa cells transfected with Env with HeLa cells transfected with CD4 (and spontaneously expressing CXCR4) results in the formation of multinuclear giant cells, which undergo apoptosis after a latency period of 24–48 h.[47] In most cases, apoptosis occurs without features of mitotic catastrophe.[48] Formation of HeLa Env/CD4 syncytia is accompanied by a progressive accumulation of cyclin B in the cytoplasm.[49] Enzymatic assays performed on syncytium-derived cyclin B-dependent kinase-1 (Cdk1) indeed reveal a transient increase in the enzymatic activity of Cdk1, peaking around 12 h after the initial fusion event.[49] Thereafter, cyclin B translocates to the nucleus, a phenomenon which occurs at the same time or shortly before the dissolution of the nuclear envelope. This process is accompanied by depolymerization of lamins and thus resembles the first step of the mitotic prophase. The dissolution of the nuclear envelope (which also can be induced by microinjection of active cyclin B/Cdk1 complexes)[49] culminates into nuclear fusion (also called 'karyogamy'), as indicated by a loss of nuclear contours within the heterokaryon.[50] At this stage, mTOR translocates to the nucleus and phosphorylates p53, causing its transcriptional activation.[51,52] Thus, a signal transducing cascade involving transcriptional activation of p53 by mammalian target of rapamycin (mTOR), p53-mediated upregulation of pro-apoptotic Bax protein, and Bax-mediated permeabilization of mitochondrial membranes participates in syncytial cell death, both in HeLa Env/CD4 syncytia and in lymphocytes infected by HIV-1.[51,53] How the Cdk1/cyclin B complex is activated in Env-induced syncytia or in HIV-1 infected patients is an ongoing conundrum. Nonetheless, it is clear that inhibition of Cdk1, either by transfection with DN-Cdk1[49] or by treatment with the pharmacological Cdk1 inhibitor roscovitine (Figure 3) can prevent the apoptosis of syncytia elicited by the interaction between HIV-1 Env and CD4.

Vpr, another pro-apoptotic protein encoded by HIV-1, has been shown to arrest cell cycle in G2 due to an inhibition of Cdk1. Thus, the transfection-enforced expression of Vpr reportedly causes Cdk1 to remain in the phosphorylated, inactive state,[54] an observation that, however, has not been confirmed by other authors.[55] Coexpression of a constitutively active mutant Cdk1 molecule with Vpr relieved the G2 arrest,[54] indicating that Vpr might induce apoptosis via its capacity to inhibit Cdk1 and to arrest the cell cycle in the G2 phase. However, a number of mutants of Vpr (e.g. truncation at C81) have been described to induce apoptosis without causing a G2 arrest,[56,57] indicating that the two functions can be uncoupled.[58] Accordingly, the addition of Vpr has no peculiar effect on syncytial apoptosis (as would be expected if it aggravated the cell cycle perturbation).[59] Rather, current observations are in line with the hypothesis that Vpr induces apoptosis through a mitochondrial pathway not related to Cdk1.[59,60] It will be interesting to follow the cell cycle characteristics of peripheral T cells from HIV-1-infected long-term non-progressors lacking the apoptogenic domain of Vpr, either due to a point mutation (R77Q)[61,62] or due to stop mutations.[57]

Conclusions

Cdk1 appears to be a major player in the homeostatic control defining the frontier between normal cellular replication, repair after damage, mitotic catastrophe and apoptosis. Clearly, uncontrolled Cdk1 activation can lead to a premature attempt of mitosis with catastrophic consequences and subsequent

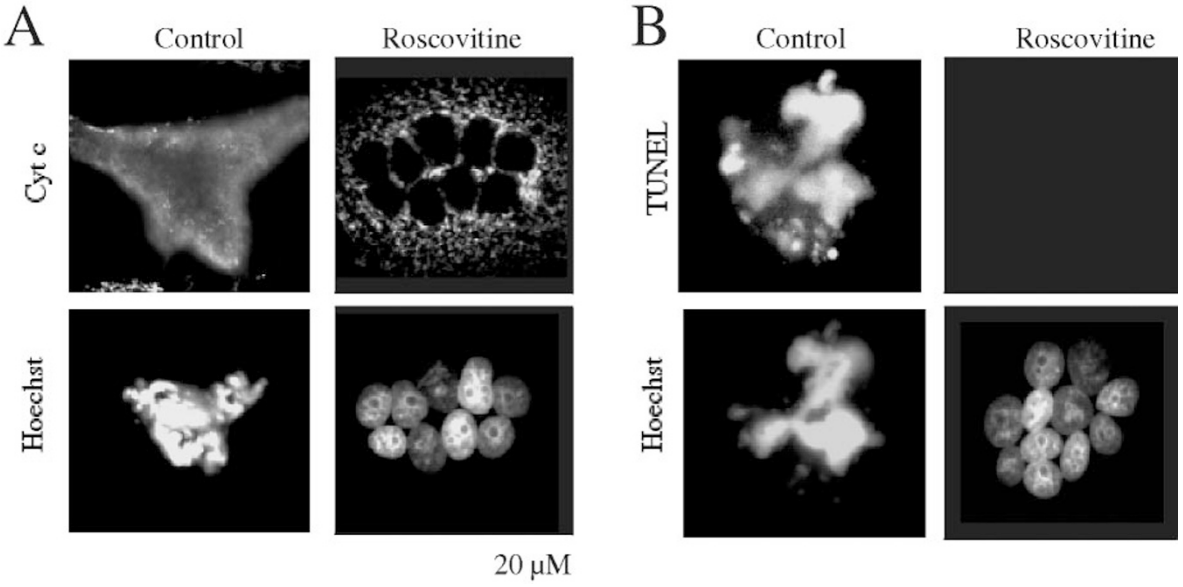

Figure 3 Cdk1-dependent apoptosis in syncytia elicited by the interaction between HIV-1 Env and CD4. HeLa cells expressing either HIV-1 Env or HIV-1 CD4 were co-cultured in the absence (control) or presence of roscovitine, a chemical Cdk1 inhibitor (10 μM, 48 h), followed by immunostaining for the detection of cytochrome *c* (**A**) or TUNEL staining (**B**) and counterstaining with Hoechst 33324 (**A, B**). Representative images show that roscovitine maintains cytochrome c in mitochondria and prevents DNA degradation

apoptosis. Cdk1 can also stimulate the activation of the apoptotic pathway in a direct fashion, through an action on Bcl-2 family proteins. The abnormal activation of Cdk1 is likely to be involved in the unwarranted cell loss accompanying HIV-1 infection and neurodegenerative disease. In addition, the failure of Cdk1 activation may determine the abnormal resistance of cancer cells to chemotherapy. In this context, it will be important to develop new strategies for the cell type-specific activation or inhibition of Cdk1 with the ultimate goal to correct disease-relevant imbalances in apoptosis control.

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