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Non-canonical functions of cell cycle cyclins and cyclin-dependent kinases

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Abstract

The role of cyclins and their catalytic partners, the cyclin-dependent kinases (CDKs), as core components of the machinery that drives cell cycle progression is well established. Increasing evidence indicates that mammalian cyclins and CDKs also carry out important roles in other cellular processes such as transcription, DNA damage repair, the control of cell death, differentiation, the immune response and metabolism. Some of these non-canonical functions are performed by cyclins or by CDKs, independent of their respective cell cycle partners, suggesting a substantial divergence in the function of these proteins during evolution.

Introduction

Progression through the cell division cycle is driven by cyclins, which bind and activate their catalytic partners, the cyclin-dependent kinases (CDKs). Specific heterodimeric cyclin–CDK complexes phosphorylate a plethora of cellular proteins to promote entry into the cell cycle and G1 phase, regulate DNA synthesis (during the S phase) and trigger segregation of the newly duplicated chromosomes to the daughter cells during mitosis, thereby ensuring that the cell cycle progresses in an ordered manner^{1, 2}.

Mammalian cyclin and CDK families each contain more than 20 members³, but only a few cyclin–CDK complexes are known to directly participate in the cell division cycle (Figure 1). Growth factors induce the expression of D-type cyclins (D1, D2 and D3), which are therefore regarded as molecular links between the cell environment and the core cell cycle machinery. Once induced, D-cyclins interact with CDK4 or CDK6, and phosphorylate the retinoblastoma protein (pRB), p107 and p130 pocket proteins, which bind and regulate E2F transcription factors during the G1 phase of the cell cycle. Later during G1, E-type cyclins (cyclins E1 and E2) become upregulated and activate CDK2 (and, to a lesser extent, CDK1 and CDK3), resulting in phosphorylation of a broader range of cell cycle-related proteins. The subsequent induction of cyclin A2 in S phase, which partners with CDK2 and CDK1,

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and activation of cyclin B1-CDK1 at the onset of mitosis drives the progression of cells through the remainder of the cell cycle through phosphorylation of a large number of proteins involved in DNA replication, as well as centrosome and chromosome function^{1, 2, 4, 5}.

Consistent with a role for cyclin-CDK complexes in promoting cell cycle progression, amplification or genetic rearrangements of cyclin or CDK genes, and/or their overexpression have been documented in nearly all human tumour types⁶⁻⁹. In particular, the cyclin D1 gene (*CCND1*) represents the second most frequently amplified locus across all types of human cancers. For these reasons cyclin-CDK complexes are considered promising targets for cancer therapy.

Although the canonical role of cyclins and CDKs as essential drivers of cell cycle entry and progression has been firmly established (Figure 1), research carried out over the past two decades provides increasing evidence for additional functions of these proteins in gene transcription, DNA damage repair, cell death, cell differentiation and metabolism. In this Review, we focus on the main biological areas where such non-canonical functions of cyclins and CDKs have been documented. We restricted our review to interphase cyclins and CDKs, that is, proteins operating in the G1, S and G2 phases. Non-cell cycle CDKs such as CDK5, are only discussed in the context of their interactions with interphase cyclins, such as cyclin E-CDK5 function in postmitotic neurons.

Kinase-dependent roles in transcription

In the current model of cell cycle regulation, the main function of cyclin D-CDK4/6 complexes is to phosphorylate and inactivate pocket proteins, thereby enabling the transcription of genes required for cell cycle progression^{2, 5, 10}. In addition, cyclin D-CDKs were shown to phosphorylate several transcription factors, thereby linking cell cycle and transcription (Table 1). Cyclin D-CDK4 complexes phosphorylate SMAD3, which inhibits the anti-proliferative effect of signalling through the transforming growth factor β pathway¹¹ (Figure 1). Cyclin D-CDK4 and cyclin D-CDK6 complexes activate the transcription factor forkhead box protein M1 (FOXM1), which regulates the expression of multiple cell cycle regulators, including proteins that govern the G2-M transition¹². Cyclin D1-CDK4-mediated phosphorylation of methylome protein 50 increases the activity of the chromatin modifier PRMT5 (protein arginine N-methyltransferase 5), which is thought to mediate key events associated with cyclin D1-CDK-dependent neoplastic growth¹³.

Also E-type and A-type cyclins have been implicated in directly modulating transcription through CDK-dependent phosphorylation of several DNA-binding factors, including SMAD3 and FOXM1^{11, 14}, ID2, upstream binding factor (UBF), and nuclear factor Y (NF-Y)¹⁵⁻²⁰ (Figure 1). Cyclin E/A-CDK2 complexes also phosphorylate the transcription factors MYC and B-MYB, thereby enhancing the ability of these proteins to activate target genes during the cell cycle^{21, 22}. In tumours driven by c-MYC overexpression, CDK2 activity seems to be essential for preventing senescence and allowing immortalization of cancer cells²³. However, while the functional link between cyclin-CDK kinases and pocket

proteins has been firmly established, the contribution of these additional phosphorylation events to normal cell cycle progression remains unknown.

Kinase-independent transcriptional roles

In addition to the role of cyclin-CDK complexes in phosphorylating transcriptional regulators, there is substantial evidence for transcriptional functions of cyclins not involving kinase activity. Interestingly, reports of cell cycle-independent transcriptional roles of cyclins and CDKs have so far been vastly dominated by D-type cyclins.

D-type cyclins have direct roles in transcription

The first indication of a pRB-independent role for cyclins in transcriptional control came from the demonstration that ectopic expression of cyclin D1 abrogated the ability of the myogenic master transcription factor MyoD to activate the muscle creatine kinase (MCK) promoter²⁴. In skeletal myoblasts, cyclin D1 recruits CDK4 to MyoD and thereby blocks MyoD-dependent transcription independently of CDK4 catalytic activity^{25–27}. Cyclin D1 was also shown to repress Sp1-mediated transcription by binding to the TATA box binding protein (TBP)-associated factor TAFII250 in a pRB-independent manner^{28, 29}. A direct interaction between cyclin D1 and the estrogen receptor has been reported to augment estrogen receptor-driven transcription in a CDK kinase-independent manner. Specifically, cyclin D1 was shown to function as a scaffold between the estrogen receptor and members of the steroid receptor coactivator family^{30, 31}, as well as between the estrogen receptor and the chromatin modifier histone acetyltransferase KAT2B (also known as P/CAF)³². In contrast, cyclin D1 and cyclin D3 inhibit the transactivation activity of the androgen receptor, another member of the nuclear steroid hormone receptor family, in a kinase-independent manner, by competing with it for P/CAF binding^{33–35,36}.

Numerous reports have subsequently described interactions of D-type cyclins with many transcription factors (Table 1). Several groups have demonstrated, mostly using protein overexpression systems, that D-type cyclins interact with sequence-specific transcription factors and activate or repress transcription by recruiting chromatin modifiers to gene promoters (or, in some cases, by blocking their access). Cyclin D1 directly binds and inhibits histone acetyltransferases such as p300 and CREB-binding protein (CBP) (in addition to P/CAF) and represses their ability to activate multiple promoters including that of the gene encoding peroxisome proliferator-activated receptor γ (PPAR γ), a master regulator of adipogenesis^{32, 37}. Consistent with these data, mouse embryonic fibroblasts lacking cyclin D1 display increased PPAR γ activity and are prone to adipogenic differentiation³⁸. Cyclin D1 can also repress transcription by neurogenic differentiation factor 1 (NeuroD) or runt-related transcription factor 3 (RUNX3), independently of CDK activity, by preventing the interaction of these transcription factors with p300^{39, 40}. Furthermore, cyclin D1 can inhibit the transcriptional activity of thyroid hormone receptors by recruiting histone deacetylases to these transcription factors and by promoting the formation of transcriptional repressive complexes⁴¹. Cyclin D2 and cyclin D3 can also modulate the activity of several transcription factors by interacting directly with them. A yeast two-hybrid screen identified the Myb-like transcription factor DMP1 as a cyclin D2

interactor⁴². Enforced expression of any D-type cyclin inhibits the transcriptional activity of DMP1 in a CDK-independent manner^{42, 43}. Intriguingly, DMP1 regulates the expression of p19^{ARF}, an inhibitor of MDM2, a negative regulator of p53, raising the possibility that D-cyclins can modulate both the pRB pathway (through CDK activity) and the p53 pathway (through CDK-independent activity).

Cyclin D3 was reported to interact with and enhance the transactivation of the vitamin D receptor, and this effect was diminished by overexpression of CDK4 or CDK6⁴⁴. Additionally, cyclin D3 interacts with human activating transcription factor 5 (hATF5) and enhances its activity⁴⁵. On the other hand, cyclin D3 competes with core binding factor β for binding RUNX1 (also known as AML1), thereby reducing the ability of RUNX1 to bind DNA and activate transcription⁴⁶.

Collectively, these reports suggest that D-cyclins might exert transcriptional effects independent of any associated kinase activity. However, the biological significance of these findings remained unclear. To address this point, human cancer cells were engineered to overexpress wild type cyclin D1 or a mutant cyclin D1 that is unable to activate CDK4 and CDK6⁴⁷. Surprisingly, the set of target genes that were induced was identical in both cases and was lacking E2F target genes, indicating that cyclin D1 plays a kinase- and E2F-independent role in transcription. Further computational analysis implicated the transcription factor CCAAT/enhancer-binding protein β (C/EBP β) as the effector of cyclin D1 in regulating gene expression. Proteomic studies using a knock-in mouse strain expressing a tagged version of cyclin D1 in place of the wild-type protein revealed that cyclin D1 interacts in vivo with several proteins involved in transcription⁴⁸. Furthermore, chromatin immunoprecipitation coupled to microarray (ChIP-on-chip) analysis revealed an extensive interaction of cyclin D1 with the mouse genome⁴⁸. In particular, within developing mouse retinas, cyclin D1 physically occupies the *Notch1* gene enhancer, to which it recruits CBP acetyltransferase, thereby activating gene expression. Genetic ablation of cyclin D1 decreased recruitment of CBP to the *Notch1* gene enhancer, and diminished histone acetylation and expression of Notch1 in the retina. Given an essential role for Notch1 in retinal development⁴⁹, these findings likely explain, at least in part, retinal developmental abnormalities seen in cyclin D1-null animals^{50, 51}. A direct interaction of cyclin D1 with chromatin was also demonstrated by a ChIP-sequencing study in which tagged cyclin D1 was ectopically expressed in cyclin D1-null mouse embryonic fibroblasts. The authors concluded that cyclin D1 plays a role in chromosomal stability through binding to genes that govern chromosomal integrity⁵².

Direct transcriptional functions of CDK6

Although the majority of reports have highlighted a CDK4/6-independent function for D-cyclins in regulating transcription, some intriguing studies revealed a kinase-independent role for CDK6 itself in this process. CDK6 was shown to physically interact with and inhibit transcriptional activity of RUNX1 in a kinase-independent fashion, and by doing so block myeloid differentiation⁵³. CDK6, but not CDK4, activates c-JUN and signal transducer and activator of transcription 3 (STAT3) to induce transcription of cyclin-dependent kinase inhibitor 2a (*Cdkn2a*; encoding the CDK4/6 inhibitor p16^{INK4A}), and *Vegfa* (encoding

vascular endothelial growth factor A, VEGF-A). Whereas the action of CDK6 on p16^{INK4A} expression requires the presence of a D-type cyclin, the effect on VEGF-A expression is independent of cyclin D⁵⁴. Interestingly, cyclin D1 has also been postulated to contribute to *VEGFA* gene transcription⁵⁵, suggesting that both CDK6 and cyclin D1 might regulate angiogenesis through different routes. The observation that CDK6 upregulates its own inhibitor p16^{INK4a} indicates the presence of a negative feedback loop, by which CDK6 safeguards against uncontrolled proliferation triggered by CDK6 overexpression⁵⁴. CDK6 can exert its pro-proliferative role only upon silencing of the p16^{INK4a} gene, an event frequently seen in human tumors⁶. Additional data suggest that the transcriptional activity of CDK6 might also be crucial in regulating the balance between quiescence and proliferation in haematopoietic and leukaemic stem cells by modulating the activity of the transcription factor early growth response protein 1 (EGR1)⁵⁶, although whether CDK6 binds it directly is currently unknown.

Cyclins and CDKs in DNA damage repair

DNA double-strand breaks (DSBs) are repaired by two distinct mechanisms: the high fidelity homologous recombination, which uses the sister chromatid as a major template for repair, and non-homologous end joining (NHEJ), which directly joins the broken DNA and is therefore error-prone. Work in several systems indicates that components of the core cell cycle machinery have important and direct roles in DSB repair.

Yeast

In *Saccharomyces cerevisiae*, inhibition of the sole cyclin-dependent kinase, Cdc28 (which is homologous to CDK1) after the induction of DSBs cripples end resection of damaged DNA, thereby preventing the loading of the homologous recombination repair proteins Rad51 and replication protein A to sites of DNA damage^{57, 58}; when Cdc28 is inhibited before the induction of DSBs, the incidence of error-prone NHEJ increases substantially, indicating that the kinase activity of Cdc28 is necessary to support homologous recombination⁵⁷ (Figure 2a). In *Schizosaccharomyces pombe*, the CDK1 homologue, Cdc2, promotes the formation of Rad51 foci, resulting in the formation of recombination intermediates at early stages of homologous recombination. At a later stage of repair, Cdc2 and a checkpoint protein Crb2 were shown to control topoisomerase III activity, thereby ensuring complete processing of recombination intermediates⁵⁹. *S. cerevisiae* Cdc28 can also regulate DNA damage repair by phosphorylating multiple substrates, such as Sae2, an endonuclease required for the initiation of resection of single-stranded DNA⁶⁰. Another substrate of the *S. cerevisiae* Cdc28 is the Srs2 helicase, the phosphorylation of which prevents its sumoylation and targets it to dismantle specific DNA structures, such as D-loops, in a helicase-dependent manner during homologous recombination⁶¹. During DNA damage repair Cdc28 also phosphorylates the G2 checkpoint protein Rad9^{62–64} and the resection nuclease Dna2⁶⁵. Collectively, these studies present a strong case for the requirement of CDK activity in the resection of DSB-ends and in mediating the choice between the homologous recombination and NHEJ repair pathways in yeast⁶⁶.

Mammals

Contrary to yeast, cyclin A- and cyclin B-associated CDK activity was found to inhibit homologous recombination in mammalian cells. Cyclin A–CDK1/2 and cyclin B–CDK1 complexes phosphorylate Ser 3291 of BRCA2⁶⁷, thereby preventing BRCA2 from binding to RAD51 and from recruiting it to sites of DNA damage (Figure 2b). DNA damage leads to reduced levels of Ser3291 phosphorylation and restoration of the interaction between BRCA2 and RAD51⁶⁷. Phosphorylation of Ser3291 is low in S-phase but increases towards mitosis, suggesting that termination of homologous recombination by CDKs is necessary for normal chromosome segregation.. Consistent with this notion, expression of mutated forms of BRCA2 that cannot be phosphorylated by CDK does not change the rate of homologous recombination, but delays the onset of mitosis⁶⁸. Conversely, CDK2 was also shown to support homologous recombination by phosphorylating CtBP-interacting protein (CtIP, the human homologue of Sae2), thereby promoting its interaction with BRCA1 and the MRE11 exonuclease and leading to the resection of DSBs ⁶⁹ (Figure 2b).

As a consequence of DNA damage, cyclin D1 is degraded and cyclin D–CDK4/6 complexes are disrupted, thereby contributing to cell cycle arrest⁷⁰. Expression of degradation-resistant mutants of cyclin D1 results in increased levels of DNA damage, confirming the importance of preventing cell cycle progression in these conditions⁷¹. An unexpected direct role for cyclin D1 in DNA damage repair was first suggested by the observation that targeting cyclin D1 to chromatin led to co-recruitment of RAD51⁷². Subsequently, it was reported⁷³ that cyclin D1 localizes, in a BRCA2-dependent manner, to irradiation-induced DSBs in human cancer cells, where it helps to recruit RAD51, thereby stimulating homologous recombination (Figure 2b). Depletion of cyclin D1 in human cancer cells reduced the recruitment of RAD51 and decreased homologous recombination, and rendered cells more sensitive to DNA damage. Notably, this function of cyclin D1 was shown to be kinase independent and to take place also in pRB-deficient cells⁷³.

A positive role for cyclin D1 in DNA damage repair might seem at odds with the observation that cyclin D1 becomes rapidly degraded after DNA damage. However, a relatively small pool of cyclin D1 persists after irradiation, and cyclin D1 becomes redistributed to DNA damage sites. This DNA-bound pool of cyclin D1 likely plays a positive role in DNA damage repair⁷³. On the other hand, forced overexpression of cyclin D1 might override cell cycle arrest following DNA damage, leading to DNA replication stress and, ultimately, apoptosis. Consistent with this notion, overexpression of cyclin D1 in cultured tumour cells rendered the cells more sensitive to radiation and triggered apoptosis^{74, 75}. One important and unresolved issue is how overexpression of cyclin D1, which is seen frequently in human cancers, affects the response of tumour cells to DNA damage *in vivo*.

Balancing cell proliferation and death

The regulation of cell cycle is tightly linked to the control of cell death. Indeed, early studies indicated that the pRB–E2F pathway can modulate the expression of multiple pro- or anti-apoptotic proteins^{5, 76}. Overexpression of cyclins in cultured cells leads to either increased apoptosis or increased survival depending on the cellular setting. For instance, ectopic

expression of cyclin D1 induces cell death and increases sensitivity to cytotoxic agents in a variety of cell lines^{74, 77–81}. By contrast, overexpression of cyclin D1 protects fibrosarcoma cells from cell death⁸², and suppression of cyclin D1 using antisense oligonucleotides sensitizes pancreatic cancer cells to apoptosis induced by cytotoxic agents⁸³. Similarly, ectopic expression of cyclin D3 in leukemic T-cells prevents them from entering apoptosis normally induced by phorbol myristate acetate⁸⁴, and ablation of cyclin D3 in T-cell acute leukemia cells triggers cell death⁸⁵. Whether these effects are direct or mediated by the E2F–pRB pathway is unclear in most of the cases. However, D-type cyclins and CDKs can also modulate apoptotic cell death through direct interaction with the apoptotic machinery. In mantle cell lymphoma cyclin D1, which is frequently overexpressed in this tumor type, binds and sequesters the pro-apoptotic protein BAX in the cytoplasm, therefore inhibiting apoptosis⁸⁶ (Figure 3). CDK4 interacts with the apoptosis inhibitor survivin (also known as baculoviral IAP repeat-containing protein 5). As a result, p21^{Cip1} is released from its complex with CDK4 and interacts with mitochondrial procaspase 3 to suppress Fas-mediated cell death⁸⁷.

A pro-apoptotic function for cyclin D3 is suggested by the observation that it can physically interact with caspase 2, leading to the stabilization of this protease and promoting its cleavage into an active form⁸⁸ (Figure 3). Cyclin D1-CDK kinase activity might play a role in mediating cell death under pathological conditions. Increased activity of cyclin D1-CDK4 correlating with neuronal death, has been reported in a rat model of stroke⁸⁹. Moreover, neuronal cell death triggered by treatment of rats with kainic acid can be prevented by injection of CDK4 or cyclin D1 antisense-oligonucleotides into brain ventricles⁹⁰. Administration of flavopiridol, a pharmacological inhibitor of CDKs into brain ventricles, prevents neuronal cell death in a model of focal ischemia⁸⁹.

Analyses of mice lacking D-type cyclins support the pro-survival function of these proteins in select tissues. Genetic ablation of cyclin D1 leads to increased apoptosis of photoreceptor cells in postnatal animals⁹¹. Whereas constitutive, germline ablation of cyclin D1 (or cyclin D2 or D3) does not cause obvious cell death in other organs, an acute shutdown of all three D-type cyclins together in bone marrow of adult mice triggers massive cell apoptosis. In this compartment D-cyclins repress the expression of a death receptor Fas and its ligand, FasL (Figure 3); ablation of the three D-cyclins results in upregulation of Fas and FasL proteins leading to hematopoietic cell apoptosis⁹².

Evidence for a direct role of cyclin E–CDK2 or cyclin A–CDK2 complexes in control of cell death is more limited. Cyclin E–CDK2 affects the apoptotic response through phosphorylation of the transcription factor forkhead box protein O1 (FOXO1), thus upregulating the expression of the pro-apoptotic proteins FAS and BIM (also known as Bcl2-L-11)⁹³. On the other hand, cyclin E–CDK2 complexes were suggested to promote survival by directly phosphorylating the anti-apoptotic factor MCL1⁹⁴ (Figure 3).

In addition to apoptosis, cyclins and CDKs can also modulate other cell death pathways. Cyclin D1 expression suppresses autophagy in the mammary epithelium⁹⁵, a function that might be mediated by the pRB–E2F pathway⁹⁶. In prostate cancer cell lines, cyclin D1 was

postulated to inhibit anoikis by binding to FOXO1 and FOXO3a, and blocking their ability to induce anoikis in a CDK-independent manner⁹⁷ (Figure 3).

Control of cell differentiation

Terminal differentiation is usually coupled to permanent exit from the cell cycle. The levels of cyclins typically decline when cells exit the cell cycle and undergo differentiation. Moreover, induction of the expression of CDK inhibitors during cell differentiation prevents activation of cyclin-CDK complexes in terminally differentiated cells (Box 1). On the other hand, expression of cyclin-CDK complexes in proliferating cells inhibits pRB function, thereby promoting proliferation and inhibiting differentiation. Indeed pRB, in addition to its well-established cell cycle role, binds and regulates the activity of several cell-type-specific transcription factors, including MyoD, myocyte-specific enhancer factor 2 (MEF2), RUNX2 and many others, thereby linking cell cycle arrest and differentiation⁵.

Box 1

CDK inhibitors

Two different families of proteins can inhibit CDK activity¹⁵⁹. Members of the CIP/KIP (CDK interacting protein/Kinase inhibitory protein) family of inhibitors — p21 (CIP1), p27 (KIP1) and p57 (KIP2), encoded by the cyclin-dependent kinase inhibitor 1 (*CDKN1*) genes — bind most cyclin-CDK complexes and prevent their activation. *CDKN2*-encoded p16 (INK4A), p15 (INK4B), p18 (INK4C) and p19 (INK4D) proteins, which constitute the INK4 family, bind CDK4 and CDK6 and block their association with the D-type cyclins. Owing to their inhibition of CDKs and cell proliferation, CDK inhibitors are tumour suppressors, and deletion of the *CDKN2A-CDKN2B* locus, which encodes p16, p15 and another protein, p14 (ARF) involved in the activation of the p53 tumour suppressor, is one of the most frequent mutation in human tumours¹⁶⁰.

CDK inhibitors are involved in many cellular processes, including cell cycle control, transcription, cell migration and metabolism; and they can affect cell quiescence, senescence, differentiation or cell death, depending on the cellular setting^{112, 143, 155, 161–164}. In most cases, these functions are mediated by inhibition of specific cyclin-CDK complexes. However, INK4 or CIP/KIP proteins can have additional activities that are unrelated to their function as CDK inhibitors¹⁶⁵. For example, p19 (INK4D) has been proposed to participate in DNA damage repair and senescence in a CDK-independent manner through directly interacting with chromatin^{166, 167}. p21 (CIP1) can function as a Rho-kinase (ROCK) inhibitor, and p57 (KIP2) modulates the subcellular localization of LIM kinase (LIMK), a serine/threonine kinase involved in regulation of actin filaments¹⁶⁸. Similarly, p27 (KIP1) has an important role in the control of cell cytoskeleton and migration¹⁶⁹ by physically interacting with RhoA and by blocking its activation, resulting in impaired stabilization of actin fibres and increased cell motility^{170,171}.

Cyclins and CDKs have also been shown to carry out direct, pRB-independent roles in cell differentiation. For example, cyclin D1-CDK4 complexes phosphorylate and target for

degradation RUNX2, a transcription factor that drives osteoblast differentiation, thereby inhibiting bone differentiation⁹⁸. CDK1 and CDK2 can likely affect cell differentiation through phosphorylation and activation of EZH2, the catalytic subunit of the histone Lys methyltransferase Polycomb repressive complex 2 (PRC2). PRC2 is an important regulator of gene expression by catalyzing the trimethylation of histone H3 Lys 27 (H3K27me3), and it influences differentiation by repressing lineage-specific genes^{99, 100}.

Roles of cyclins and CDKs in muscle differentiation

As mentioned above, cyclin D1 and CDK4 can prevent transcription activation mediated by MyoD, and hence inhibit myoblast differentiation^{24–27, 101} (Figure 4a). Although this activity is partially kinase-independent, maximal repression of MyoD, as well as repression of another muscle-specific basic helix-loop-helix (bHLH) factor, myogenin, requires CDK kinase activity¹⁰². Cyclin D–CDK4 complexes also block the association of MEF2 with glutamate receptor-interacting protein 1 (GRIP1), a member of the steroid receptor family of transcription co-activators, thereby inhibiting the ability of MEF2 to induce muscle gene expression¹⁰². This block requires CDK activity, although the exact molecular mechanism mediating it is unclear¹⁰². Cyclin D1 can also inhibit the differentiation of cardiomyocytes through CDK4-dependent phosphorylation of GATA4, which targets this transcription factor for degradation¹⁰³ (Figure 4a).

By contrast, specific D-cyclins are upregulated during terminal differentiation of certain cell types. In differentiating skeletal myoblasts, the expression of cyclin D3 is strongly upregulated (over 20-fold), in part by MyoD^{101, 104, 105}. In differentiated myotubes, cyclin D3 becomes stabilized by binding to pRB¹⁰⁶ (Figure 4a). High levels of cyclin D3 have also been observed in several other terminally differentiated cell types, including many types of epithelia, oocytes and ovarian corpora lutea, podocytes within renal glomeruli, adipocytes (see below) and cells of the exocrine pancreas^{107–110}. On the basis of these observations, cyclin D3 has been postulated to carry out cell cycle-independent functions in a variety of terminally differentiated cells. However, these findings seem to be at odds with the observation that mice lacking cyclin D3 develop normal muscle and epithelia¹¹¹. This might be attributed to functional redundancy between the three D-type cyclins, and conditional knockout approaches involving deletion of all D-cyclins in postmitotic tissues might help to clarify this point.

Cyclin D–CDKs in neuronal development

Cyclin–CDK complexes can inhibit neuronal differentiation, not only through the pRB–E2F pathway¹¹² but also by phosphorylating and inactivating transcription factors that drive neurogenesis. For instance, neurogenin 2 (NGN2), a master regulator of neuronal differentiation, is inhibited by multisite phosphorylation by CDKs (Figure 4b)^{113, 114}, and a non-phosphorylatable NGN2 mutant can drive neuronal differentiation in the presence of CDK activity¹¹³. Cyclin D1–CDK4 activity in neural stem cells is also thought to inhibit neurogenesis and expand the population of basal progenitors by shortening the duration of G1, although the exact mechanism remains to be clarified¹¹⁵. In support for a role for cyclin–CDK complexes in inhibiting neuronal differentiation, embryos from *Cdk2*^{-/-}*Cdk4*^{-/-}

knockout mice lack the intermediate zone and cortical plate in their brains, due to premature neuronal differentiation and depletion of basal progenitor cells¹¹⁶.

Other data support the opposite conclusion — namely, that D-cyclins promote neurogenesis. A cell cycle-independent function for cyclins in the nervous system was first proposed based on the upregulation of cyclin D1-associated kinase activity in rat brains at the onset of neuronal differentiation^{117, 118}. High levels of cyclin D1 persisted in the brains of adult mice, whereas the levels of cyclin D2 decreased, suggesting a specific role for cyclin D1 in neuronal differentiation. By contrast, high levels of cyclin D2 were observed in the brains of adult mice, suggesting that cyclin D2 carries out a distinct role in terminally differentiated neurons¹¹⁹.

Loss-of-function and gain-of-function experiments in embryonic chick spinal cord also supported a role for cyclin D1 in promoting neuronal differentiation¹²⁰. In vivo knockdown of cyclin D1, but not cyclin D2, significantly reduced differentiation of motoneuron progenitors. Conversely, forced overexpression of cyclin D1 enhanced neurogenesis, whereas cyclins D2 and D3 inhibited this process. Interestingly, overexpression of a cyclin D1 mutant that is unable to activate CDKs promoted neurogenesis to an even higher extent than wild-type cyclin D1¹²⁰. Collectively these findings suggest a differential role for cyclins D1 and D2 in neurogenesis, but the molecular mechanism involved remains unclear.

Cyclin E in neuronal development

Cell cycle proteins also affect the balance between progenitors and neurons by controlling the regulation of asymmetric cell divisions of neural progenitors¹²¹. In *Drosophila melanogaster*, cyclin E is expressed in the thoracic cell lineage, which undergoes asymmetric divisions giving rise to neuroblasts (central nervous system stem cells in *D. melanogaster*) and to glial progenitors. Depletion of cyclin E renders the thoracic lineage capable of generating only glial progenitors, whereas ectopic expression of cyclin E in the abdominal lineage forces it into asymmetric divisions¹²². Mechanistically, cyclin E interacts with and inhibits the activity of Prospero, a transcription factor required for glial cell identity¹²³. The role of E-type cyclins in asymmetric cell divisions of mammalian neural progenitors has not been explored.

Interestingly, cyclin E is upregulated in the brains of postnatal animals as neurons undergo terminal differentiation. In terminally differentiated neurons, cyclin E forms a complex with CDK5¹²⁴ (Figure 4b), which is a well-established regulator of neuronal differentiation¹²⁵. Cyclin E sequesters CDK5 into kinase-inactive cyclin E–CDK5 complexes, away from the CDK5 activating partners p35 and p39. By this mechanism, cyclin E controls phosphorylation of key synaptic proteins and regulates synaptic plasticity and memory formation. Ablation of cyclin E in the mouse brains decreased synaptic function and rendered animals unable to normally retain memories¹²⁴. These findings raise a possibility that abnormalities in cyclin E levels might underlie some cases of human learning disabilities¹²⁴.

Immune system differentiation and function

A role for CDK2 in suppressing immune tolerance was highlighted by the observations that when cardiac allografts were transplanted into Cdk2-null recipient mice, a long-term allograft acceptance was observed, in contrast to wild-type recipients, which rejected the grafts¹²⁶. Cdk2-deficient regulatory T-cells (Treg) displayed increased suppressive capacity, likely explaining the increased immune tolerance in Cdk2-null mice^{126, 127}. Cyclin E-CDK2 was shown to phosphorylate and negatively regulate forkhead box protein P3 (FOXP3), a transcription factor required for the development and function of Treg¹²⁸. Furthermore, in developing lymphocytes, cyclin A-CDK2 triggers the periodic degradation of the recombinase RAG-2, thereby restricting V(D)J recombination to G1 phase¹²⁹. Mutation of CDK2 substrate residues of RAG-2 abrogated cell cycle-dependent degradation of this protein¹³⁰.

Regulation of cell migration

During keratinocyte differentiation, cyclin D1 co-localizes with SEC6, a subunit of the exocyst complex involved in vesicle trafficking, as well as with $\beta 1$ integrin and RalA (a GTPase that regulates the exocyst). Ectopic overexpression of cyclin D1 increases $\beta 1$ integrin recycling upon differentiation, as demonstrated by a pronounced loss of membrane-bound $\beta 1$ integrin, and leads to reduced keratinocyte adhesion to the extracellular matrix¹³¹. Cell migration and the actin cytoskeleton are also modulated by cyclins and CDKs at different levels¹³². CDK6 was demonstrated to localize to the ruffling edges of spreading fibroblasts prior to the formation of filamentous actin, and to promote migration in a $\alpha_v\beta_3$ integrin-dependent manner¹³³. Cyclin D1 was implicated in promoting fibroblast migration by inhibiting the thrombospondin 1 and Rho kinase (ROCK) signalling pathways¹³⁴. Cyclin D1 can also enhance cell migration by negatively regulating the transcription of the E3 ubiquitin ligase factor S-phase kinase-associated protein 2 (SKP2)¹³⁵. SKP2 promotes the degradation of the CDK inhibitor p27^{Kip1}, which was implicated in cell migration through control of the RhoA pathway¹³⁶ (Box 1). Cyclin D1 can also affect cell migration by binding to, and promoting the phosphorylation of filamin A, a member of the actin-binding filamin protein family. Knockdown of cyclin D1 or ectopic expression p16^{INK4A} reduced the phosphorylation of filamin A and of many other actin binding proteins, suggesting a direct role for a cyclin D1-associated kinase in regulating the cytoskeleton¹³⁷. Furthermore, it was postulated that cyclin D1, in a CDK-independent manner, mediates its effect on cell migration by transcriptionally regulating Dicer¹³⁸, which is an endoribonuclease required for microRNA processing. In summary, although cyclin D1 has been shown to promote cell migration in multiple cell types, the major mechanism responsible for this function remains unclear. It also remains to be seen whether overexpression of cyclin D1, seen in many cancer types, contributes to the increased invasiveness of tumour cells.

Roles of cyclins and CDKs in metabolism

Multiple cellular functions of cyclins and CDKs converge to control the generation of cellular energy and metabolism at both the cellular and organismal levels.

Cellular energy production and metabolism

In *D. melanogaster* and in mammalian cells, cyclin D–CDK4 activity positively regulates mitochondrial biogenesis through the pRB–E2F-dependent control of mitochondrial transcription factors, such as NRF1 and NRF2¹³⁹ (Figure 5). By contrast, cyclin D1–CDK4 can also inhibit mitochondrial function: depletion of cyclin D1 in mouse breast cancer cells *in vivo* promoted expression of genes involved in mitochondrial function and increased the number of mitochondria¹⁴⁰, and cyclin D1-null mouse mammary epithelial cells had increased mitochondrial size¹⁴⁰. The inhibitory effects of cyclin D1 on mitochondrial function were attributed to mediating the repression of NRF1 through CDK-dependent phosphorylation, leading to impaired expression of mitochondrial transcription factor A (mtTFA), a factor essential for mitochondrial respiration¹⁴¹ (Figure 5). Cyclin D1 was also shown to affect mitochondrial function by inhibiting the expression and activity of the glycolytic enzyme hexokinase 2¹⁴⁰.

Cyclin D1 can also exert its effect on mitochondria independently of CDK4 by binding to the voltage-dependent anion channel protein (VDAC) in the outer mitochondrial membrane, thereby preventing VDAC from binding to hexokinase 2 and impairing the access of ADP to the inner mitochondrial membrane¹⁴². Further observations that cyclin D1 can directly bind additional lipogenic enzymes and mitochondrial proteins⁴⁸ point to cyclin D1 as an important regulator of metabolic activities through different pathways¹⁴³. In addition to cyclin D1, also cyclin E has been proposed to inhibit mitochondrial biogenesis and oxidative metabolism in a transcriptional manner, but the exact mechanism is unknown¹⁴⁴. Lastly, the cyclin B1–CDK1 complex was shown to localize to the matrix of mitochondria, where it phosphorylates multiple mitochondrial proteins such as the respiratory chain complex 1 subunits, leading to increased mitochondrial respiration. It was postulated that this event coordinates mitochondrial respiration with G2–M phase progression¹⁴⁵.

Regulation of metabolism at the organismal level

A physiological role for cell cycle proteins in metabolism was first documented by the observations that mice lacking CDK4 developed diabetes due to the loss of pancreatic islet-beta cells^{146, 147}. Conversely, knock-in mice expressing a constitutively active CDK4 mutant developed pancreatic hyperplasia¹⁴⁶. Mice lacking cyclin D2 also displayed substantially reduced beta cell mass and reduced islet size, leading to glucose intolerance¹⁴⁸. This phenotype was further exacerbated by the additional heterozygous deletion of cyclin D1, and cyclin D1^{+/-}D2^{-/-} mice developed life-threatening diabetes by 3 months of age¹⁴⁹. Ablation of cyclin D3 exacerbated diabetes in non-obese diabetic mice, but this did not affect the proliferation rate of beta cells, raising the possibility that cyclin D3 might have a cell cycle-independent role in this tissue¹¹⁰. Glucose challenge was proposed to promote cyclin D2–CDK4 complex formation and kinase activity, which indirectly induces the expression of Kir6.2, a component of the potassium ATP channel that positively regulates insulin secretion (Figure 5)¹⁵⁰. Conversely, inhibition of CDK4 activity leads to lower expression of Kir6.2, decreases insulin secretion and results in glucose intolerance¹⁵⁰. The effect of cyclin D2–CDK4 activity on Kir6.2 levels is mediated by E2F1, but it occurs independently of cell cycle progression¹⁵⁰. An independent mechanism linking insulin signalling to cyclin D1–CDK4 was provided by the observation that insulin-mediated upregulation of cyclin D1, and

subsequent activation of cyclin D1–CDK4 in hepatocytes leads to phosphorylation and activation of the histone acetyltransferase GCN5 (also known as KAT2A). GCN5 is responsible for the acetylation and inhibition of the transcription factor PPAR γ coactivator 1 α (PGC1 α)¹⁵¹, which inhibits the expression of gluconeogenesis genes, thereby suppressing hepatic glucose production¹⁵¹ (Figure 5). Cyclin D1 was shown to inhibit lipid metabolism in the liver, in a kinase-independent manner, through its interaction with and suppression of the transcription factor hepatocyte nuclear factor 4 α ¹⁵².

The functions of cyclins and CDKs in modulating metabolic pathways contribute to the ability of these proteins to affect differentiation. A positive role for cyclin D3 in promoting adipocyte differentiation is supported by the observation that knockdown of cyclin D3 inhibits adipogenesis *in vitro*¹⁵³. Consistent with these data, mice lacking cyclin D3 or CDK4 are protected from diet-induced obesity, have smaller adipocytes and reduced expression of adipogenesis genes^{109, 154}. CDK4 — likely in complex with cyclin D3 — also participates in adipocyte differentiation by directly interacting with and activating PPAR γ , the master regulator of adipogenesis^{109, 154} (Figure 5). Furthermore, cyclin D–CDK4 complexes may engage the pRB–E2F pathway to inhibit the pRB-mediated repression of PPAR γ ¹⁵⁵. Cyclin D1, on the other hand, was shown to inhibit adipogenesis by inhibiting p300 and recruiting histone deacetylases to PPAR response element of the lipoprotein lipase promoter, and cyclin D1-null fibroblasts are prone to adipogenic differentiation^{37, 38}.

Conclusions and perspectives

Research carried out over the past two decades indicates that interphase cyclins and CDKs display functions that extend well beyond regulation of cell cycle progression. Yet, further work is needed to reconcile often conflicting models and observations, which are perhaps confounded by the overlapping and/or compensatory roles of the different cyclin and CDK proteins in mammals. Dysregulation of cell cycle proteins is commonly found in several pathological conditions including cancer, neurodegeneration and cardiac disease, but the relevance of non-canonical functions of cell cycle proteins in controlling processes other than cell proliferation — such as the control of transcription, cell death, differentiation and metabolism — remains to be fully explored.

Some of the proposed non-canonical roles might not reflect a normal physiological protein function, but a gain-of-function event that occurs in tumour cells as a consequence of cyclin and/or CDK-overexpression. Given that several inhibitors of CDK4/6 kinase activity are currently in clinical trials^{156, 157}, it will be important to elucidate which of these cell cycle-independent roles of cyclins and CDKs truly contribute to tumorigenesis *in vivo*. According to the prevailing model, the major role of CDK4 and CDK6 is to inactivate pRB. Indeed, inhibition of CDK4 and CDK6 has no effect on proliferation of tumour cells that lost pRB expression¹⁵⁸. Given the increasing number of reported CDK4/6 substrates, it remains to be seen whether CDK4/6 inhibition in pRB-negative tumour cells affects other non-canonical functions of cyclin D–CDK4/6 kinases, and, by doing so, contributes to the therapeutic effect.

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Glossary

Allografts	Transplants from genetically non-identical individuals of the same species
Anoikis	A special form of programmed cell death induced in anchorage-dependent cells by their detachment from the surrounding matrix
Apoptosis	Programmed cell death characterized by the activation of specific proteases, termed caspases, which induce chromatin fragmentation and the degradation of multiple cellular components
Asymmetric cell division	A especial form of cell division in which the daughter cells have different fates, likely as a consequence of asymmetric distribution of cellular components during division
Autophagy	The degradation and recycling of cellular components and organelles by lysosomes
Basic Helix–Loop–Helix (bHLH) transcription factor	A protein that contains two helices separated by a loop (the HLH domain), which binds DNA in a sequence-specific manner
Chromatin modifier	A protein complex that, through catalyzing specific histone or DNA modifications, induces changes in chromatin to make it accessible (open) or inaccessible (closed) to the transcriptional machinery
D-loop	Displacement loop formed in homologous recombination repair. Two strands of a homologous chromosome are separated for a stretch by an invading broken strand forming a displaced single-strand structure, a D-loop
Exocyst complex	A highly conserved, octameric protein complex that regulates vesicle delivery to and docking at the cell surface
Gluconeogenesis	Generation of glucose from non-carbohydrate carbon substrates
Quiescence	A postmitotic state in which cells do not divide, although they may be induced to re-enter the cell cycle in response to specific stimuli

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Biographies

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Online summary

- In addition to their well-established functions in driving cell proliferation, cell cycle proteins have several non-canonical roles.
- D-type cyclins and their partner cyclin-dependent kinase 6 (CDK6) have direct, kinase-independent roles in augmenting or repressing gene expression.
- In mammalian cells, cyclin D1 promotes, whereas cyclin A–CDK2 inhibits DNA double-strand break (DSB) repair through homologous recombination. In yeast, CDK activity seems to dictate the choice of DSB repair between non-homologous end joining and homologous recombination.
- Cyclins are postulated to regulate apoptosis, autophagy and anoikis. Analyses of mice lacking D-cyclins support pro-survival roles for these proteins in specific tissues.
- Cyclin D1, CDK6 and the CDK-inhibitor p27 (KIP1) can affect the actin cytoskeleton and cell migration through several different mechanisms.
- Cell cycle proteins have important roles in development and have important functions in the nervous system and in regulating the immune response.
- Cyclins and CDKs are shown or postulated to regulate metabolism through different routes, including a direct role in controlling mitochondrial function.

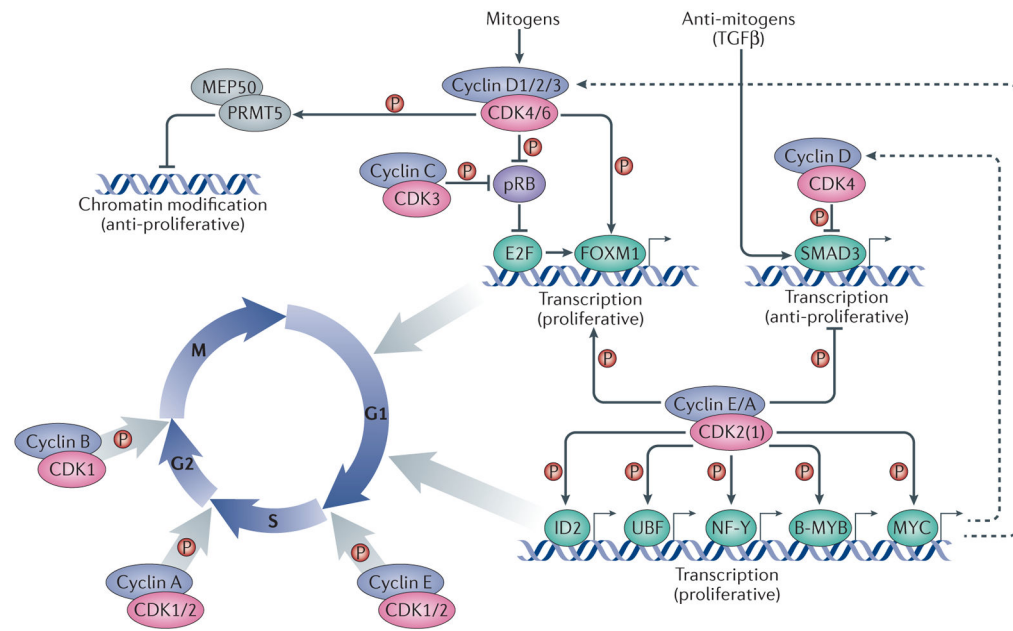


Figure 1. Canonical roles of cyclins and CDKs in the cell division cycle

Cell cycle progression is driven by heterodimeric complexes formed by cyclin D, cyclin E, cyclin A or cyclin B with cyclin-dependent kinase (CDK) 4, CDK6, CDK2 or CDK1. (Cyclin C–CDK3 complexes also participate in G0 to G1 phase progression although their physiological relevance in this process is less well established). Cell cycle entry is driven by the formation of cyclin D–CDK4/6 complexes in response to mitogenic stimulation. These complexes phosphorylate and partially inactivate the retinoblastoma protein, pRB, thereby releasing its inhibition of the transcription factor E2F. E2F subsequently promotes the expression of multiple cell cycle genes, among which are E-type cyclins, which bind to and activate CDK2 (and, to a lesser extent, CDK1). Activation of CDK2 (and CDK1) by E-cyclins and A-cyclins leads to the phosphorylation of multiple transcription factors, such as the helix-loop-helix (HLH) protein ID2 as well as UBF, NF-Y, B-MYB and MYC, which contribute to cell cycle progression at different levels. Cyclin D1–CDK4 complexes also phosphorylate chromatin modifiers, such as the protein arginine N-methyltransferase 5 (PRMT5) cofactor MEP50, to promote the repression of specific genes with anti-proliferative properties. Multiple cyclin–CDK complexes can phosphorylate the transcription factors SMAD3, which is activated by antimitogens such as TGFb, and FOXM1 to promote cell cycle progression. Entry into mitosis is specifically driven by cyclin B–CDK1 complexes. Dashed arrows indicate indirect connections or connections with multiple steps. Abbreviations: MEP50, methylome protein 50; NF-Y, nuclear factor Y; UBF, upstream binding factor.

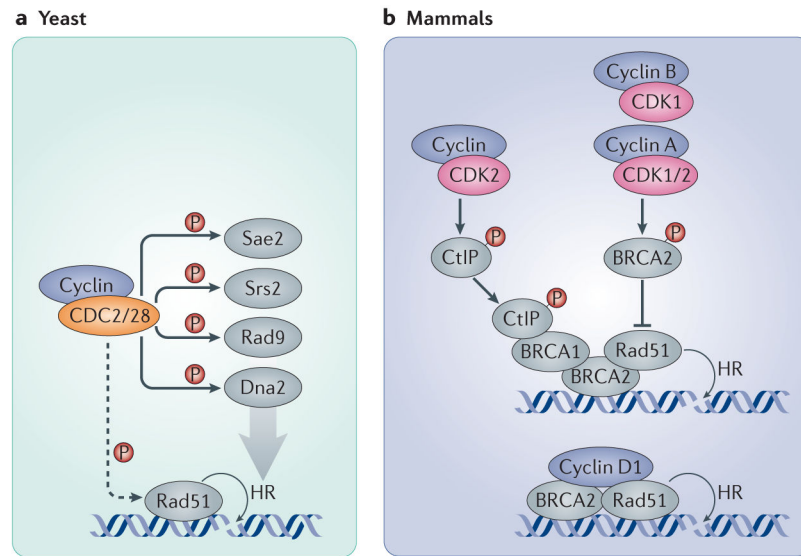


Figure 2. Cyclins and CDKs in DNA double-strand breaks (DSBs) repair

a| In yeast, the sole CDK (CDC28 in *S. cerevisiae* and CDC2 in *S. pombe*) participates in the recruitment of Rad51 to DNA double strand breaks and phosphorylates several proteins involved in the DNA damage response and the repair of DSBs through homologous recombination, such as the endonuclease Sae2, the helicase Srs2, the G2 checkpoint protein Rad9 and the resection nuclease Dna2. b| In mammalian cells, CDK2 and CDK1 (together with A-, B- and potentially also E-type cyclins) modulate homologous recombination by the direct phosphorylation of BRCA2, preventing it from binding and recruiting RAD51. Conversely, CDK2 can support homologous recombination by phosphorylating CtBP-interacting protein (CtIP; the human homologue of Sae2), which promotes the recruitment of BRCA1 to DSBs. In addition, cyclin D1 helps to recruit RAD51 to DSB sites by directly interacting with it in a CDK-independent manner, thereby promoting homologous recombination. Dashed arrows indicate indirect connections or connections with multiple steps.

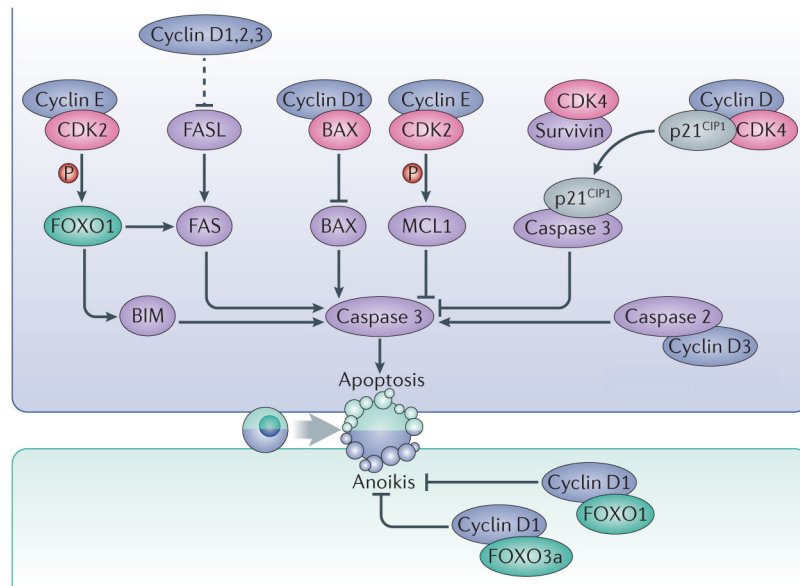


Figure 3. Regulation of cell death by interphase cyclins and CDKs

Interphase cyclins modulate apoptosis using CDK-dependent and independent mechanisms. Cyclin E–CDK2 complexes phosphorylate the prosurvival factor MCL1 as well as the transcription factor FOXO1, leading to inhibition or activation of apoptosis, respectively. Phosphorylation of MCL1 stabilizes the protein while phosphorylation of FOXO1 leads to upregulated expression of the pro-apoptotic factors FAS and BIM. Binding of survivin to CDK4 causes the release of the CDK inhibitor p21 (CIP1), which binds and inhibits caspase 3. D-type cyclins can also directly interact with the pro-apoptotic proteins BAX or caspase 2. Moreover, the three D-type cyclins have overlapping roles in repressing the expression of the death receptor FAS and its ligand, FASL, thereby preventing apoptosis in haematopoietic cells. Cyclin D1 also interacts with the transcription factors FOXO1 and FOXO3a and prevents anoikis. Dashed arrows indicate indirect connections or connections with multiple steps.

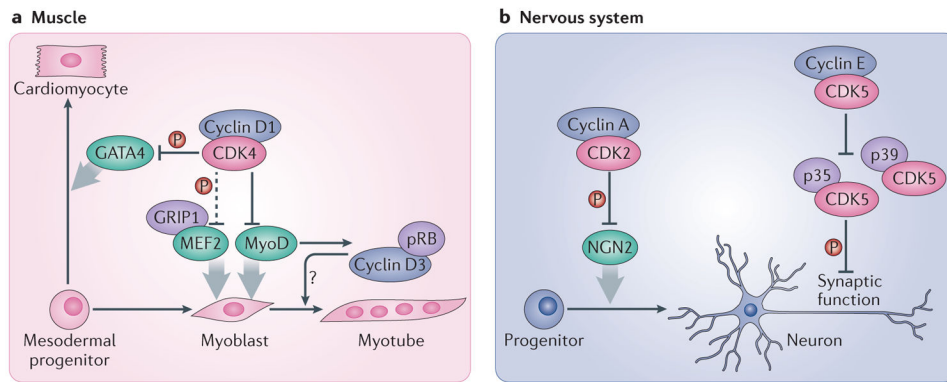


Figure 4. Roles of interphase cyclins and CDKs in cell differentiation

a| In muscle progenitor cells, cyclin D1 interacts with the transcription factor MyoD and inhibits its activity. This effect requires CDK4 but is partially independent of its kinase function. However, the catalytic activity of CDK4 is required for the inhibition by cyclin D1–CDK4 of GRIP1–MEF2 complexes, which are also involved in muscle differentiation. Cyclin D1–CDK4 complexes also phosphorylate and inactivate the GATA4 transcription factor, and thereby repress the differentiation of cardiomyocytes. On the other hand, cyclin D3, which is stabilized in differentiated muscle cells by its interaction with pRB, seems to promote muscle cell differentiation by an unknown mechanism. b| In the nervous system, the differentiation of neuronal progenitors into neurons is blocked by cyclin A–CDK2 complexes, which phosphorylate and inactivate the NGN2 transcription factor. Cyclin E regulates synaptic plasticity by forming kinase-inactive complexes with CDK5 and sequestering it from its activators p35 and p39, thereby inhibiting the phosphorylation of synaptic CDK5 substrates. Dashed arrows indicate indirect connections or connections with multiple steps.

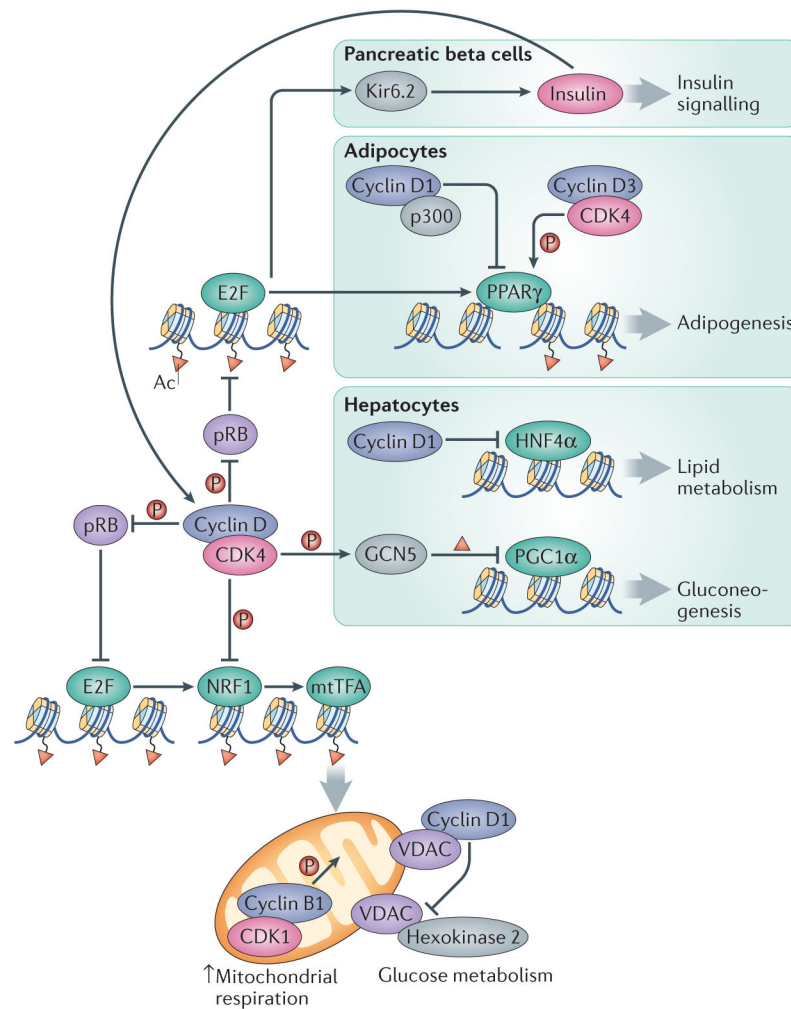


Figure 5. An integrated view of the roles of interphase cyclins and CDKs in the control of cellular energy and physiological metabolism

Cyclin–CDK complexes display opposing roles in controlling mitochondrial function.

Whereas their activity promotes the expression of the NRF1 factor through the pRB–E2F pathway, cyclin D1–CDK4 can also directly phosphorylate and inactivate NRF1, leading to decreased transcription of mitochondrial proteins by mitochondrial transcription factor A (mtTFA). Moreover, although the activity of mitochondria is directly stimulated by cyclin B1–CDK1, cyclin D1 binding to voltage-dependent anion channel protein (VDAC) prevents hexokinase 2 activation and the use of glucose in mitochondria. In pancreatic beta cells, cyclin D–CDK4 complexes promote insulin secretion through the E2F-mediated expression of the potassium ATP channel component Kir6.2. Insulin, in turn, induces the expression of D-type cyclins through a positive feedback loop. In adipocytes, adipogenesis is inhibited by the interaction of cyclin D1 with the histone acetyltransferase p300, which leads to inhibition of the transcription factor peroxisome proliferator-activated receptor γ (PPAR γ). Conversely, adipogenesis is activated by cyclin D3–CDK4-mediated PPAR γ phosphorylation. Gluconeogenesis is inhibited in hepatocytes through the cyclin D1–CDK4-dependent phosphorylation and activation of the acetyltransferase GCN5, which acetylates

and inactivates the transcription factor PPAR γ coactivator-1 α (PGC1 α). In addition, cyclin D1 can bind and inactivate the transcription factor hepatocyte nuclear factor-4 α (HNF4 α), resulting in deficient lipid metabolism.

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Table 1

Representative transcription factors regulated by interphase cyclins and CDKs*

Transcription factor	Cyclin	CDK	Kinase dependent?	Activation or Repression	Biological function(s)	Reference
Regulation by cyclin--CDK complexes						
B-Myb	A2	CDK2	YES	Activation	Promoting cell cycle progression	21
c-Myc	E1	CDK2	YES	Activation	Repression of cellular senescence	22
FOXMI1C	D1, E1, A2	CDK2, CDK4	YES	Activation	Promoting cell cycle progression, repression of cellular senescence	12, 14
FOXO1	E	CDK2	YES	Repression	Mediating genotoxic responses	93
FOXP3	E	CDK2	YES	Repression	Development of regulatory T-cells	128
GATA4	D1	CDK4	YES	Repression	Preventing cardiomyocyte differentiation	103
ID2	E1, A2	CDK2	YES	Repression	Promoting cell cycle progression	15
MEF2	D3 (D1)	CDK4	YES	Repression	Preventing muscle differentiation	102
NF-Y	A2	CDK2	YES	Activation	Promoting cell cycle progression	1718
NGN2	A-	CDK2, CDK1	YES	Repression	Inhibition of neuronal differentiation	113
NRF1	D1	CDK4	YES	Repression	Inhibition of mitochondrial function	141
PPAR γ	D3	CDK4	YES	Repression	Induction of adipogenesis	109, 154
RUNX2	D1	CDK4	YES	Repression	Inhibition of bone differentiation	98
SMAD3	-	CDK2, CDK4	YES	Repression	Preventing TGF β signaling	11
UBF	E1, A2	CDK2	YES	Activation	Ribosomal DNA transcription	16
Regulation by cyclins						
RUNX1 (AML1)	D3	-	NO	Repression	Inhibition of myeloid differentiation	46
AR	D1, D3	-	NO	Repression	Inhibition of hormone-dependent gene expression	33, 36
NeuroD	D1	-	NO	Repression	Inhibition of neuronal differentiation	39
C/EBP β	D1	-	NO	Activation	Promoting mammary epithelial differentiation	172
CBP	D1	-	NO	Activation	Promoting Notch-dependent retinal development	3648
DMP1	D2 (D1, D3)	-	NO	Repression	Modulation of the p53 pathway	42
ER	D1	-	NO	Activation	Activation of hormone-dependent gene expression	30-32
FOXO1, O3	D1	-	NO	Repression	Inhibition of anoikis	97

Transcription factor	Cyclin	CDK	Kinase dependent?	Activation or Repression	Biological function(s)	Reference
hATF5	D3	-	NO	Activation	Multiple cellular processes	45
HNF4a	D1	-	NO	Repression	Inhibition of lipogenesis in the liver	152
MYOD	D1	CDK4	NO	Repression	Blocking myoblast differentiation	24, 25, 27
PPAR γ	D1	-	NO	Repression	Blocking adipogenesis	38
RUNX3	D1	-	NO	Repression	Cell proliferation	40
SP1	D1	-	NO	Repression	Multiple cellular processes	28
STAT3	D1	-	NO	Repression	Cytokine signaling	173
TR	D1	-	NO	Repression	Hormone-dependent gene expression	41
VDR	D3	-	NO	Activation	Vitamin-dependent gene expression	44
Regulation by CDKs						
c-JUN	-	CDK6	NO	Activation	Stimulation of angiogenesis	54
RUNX1	-	CDK6	NO	Repression	Inhibition of myeloid differentiation	53
STAT3	D1,2,3	CDK6	NO	Activation	Cell cycle inhibition	54

* pRB, p107 and 130 proteins are not included.

Abbreviations: AR, androgen receptor; ER, estrogen receptor; TR, thyroid hormone receptor; VDR, vitamin D receptor; B-Myb, V-Myb Avian Myeloblastosis Viral Oncogene Homolog-Like 2; c-Myc, V-Myc Avian Myelocytomatosis Viral Oncogene Homolog; FOXM1C, Forkhead Box Protein M1C; FOXO1, Forkhead Box Protein O; FOXP3, Forkhead Box Protein P3; GATA4, GATA Binding Protein 4; ID2, Inhibitor Of DNA Binding 2, Dominant Negative Helix-Loop-Helix Protein; MEF2, Myocyte-Specific Enhancer Factor 2; NF-Y, Nuclear Transcription Factor Y; NGN2, Neurogenin 2; NR1F1, Nuclear Respiratory Factor 1; PPAR γ , Peroxisome Proliferator-Activated Receptor Gamma; RUNX2, Runt-Related Transcription Factor 2; SMAD3, SMAD Family Member 3; UBF, Upstream Binding Transcription Factor; RUNX1, Runt-Related Transcription Factor 1; NeuroD, Neuronal Differentiation; C/EBP β , CCAAT/Enhancer Binding Protein Beta; CBP, CREB Binding Protein; DMP1, Dentin Matrix Acidic Phosphoprotein 1; FOXO3, Forkhead Box Protein O3; hATF5, human Activating Transcription Factor 5; HNF4a, Hepatocyte Nuclear Factor 4 Alpha; MYOD, Myogenic Differentiation; RUNX3, Runt-Related Transcription Factor 3; SP1, Specificity Protein 1; STAT3, Signal Transducer And Activator Of Transcription 3; c-JUN, V-Jun Avian Sarcoma Virus 17 Oncogene Homolog.