

Cell cycle, CDKs and cancer: a changing paradigm

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Abstract | Tumour-associated cell cycle defects are often mediated by alterations in cyclin-dependent kinase (CDK) activity. Misregulated CDKs induce unscheduled proliferation as well as genomic and chromosomal instability. According to current models, mammalian CDKs are essential for driving each cell cycle phase, so therapeutic strategies that block CDK activity are unlikely to selectively target tumour cells. However, recent genetic evidence has revealed that, whereas CDK1 is required for the cell cycle, interphase CDKs are only essential for proliferation of specialized cells. Emerging evidence suggests that tumour cells may also require specific interphase CDKs for proliferation. Thus, selective CDK inhibition may provide therapeutic benefit against certain human neoplasias.

Unscheduled proliferation

Activation of the cell cycle machinery leading to cell proliferation in the absence or presence, respectively, of appropriate mitogenic or anti-mitogenic signalling.

Genomic instability

(GIN). A tendency of the genome to acquire mutations and chromosomal aberrations owing to dysfunctional replication or repair of the cellular genome.

Chromosomal instability

(CIN). A tendency of the genome to acquire numerical aberrations in the chromosomes when chromosome segregation during mitosis is dysfunctional. CIN is frequently seen as a form of GIN, but we consider them as separate entities for clarity.

During the past two decades, a vast body of literature has illustrated the relevance of cell cycle deregulation in human cancer (TIMELINE). Tumour cells accumulate mutations that result in constitutive mitogenic signalling and defective responses to anti-mitogenic signals that contribute to unscheduled proliferation^{1,2}. In addition, most tumours acquire genomic instability (GIN) that leads to additional mutations as well as chromosomal instability (CIN), a defect responsible for numerical changes in chromosomes^{3,4} (FIG. 1). These alterations, taken together, result not only in proliferative advantages but also in increased susceptibility to the accumulation of additional genetic alterations that contribute to tumour progression and acquisition of more aggressive phenotypes. These three basic cell cycle defects — unscheduled proliferation, GIN and CIN — are mediated, directly or indirectly, by misregulation of cyclin-dependent kinases (CDKs)⁵.

CDK activity requires binding of regulatory subunits known as cyclins. Cyclins are synthesized and destroyed at specific times during the cell cycle, thus regulating kinase activity in a timely manner. Human cells contain multiple loci encoding CDKs and cyclins (13 and 25 loci, respectively)⁵. However, only a certain subset of CDK–cyclin complexes is directly involved in driving the cell cycle. They include three interphase CDKs (CDK2, CDK4 and CDK6), a mitotic CDK (CDK1, also known as cell division control protein 2 (CDC2)) and ten cyclins that belong to four different classes (the A-, B-, D- and E-type cyclins). Tumour-associated mutations frequently deregulate certain CDK–cyclin complexes, resulting in either continued

proliferation or unscheduled re-entry into the cell cycle, two properties characteristic of most human tumour cells¹ (FIG. 1).

Proper progression through the cell cycle is monitored by checkpoints that sense possible defects during DNA synthesis and chromosome segregation. Activation of these checkpoints induces cell cycle arrest through modulation of CDK activity. Cell cycle arrest allows cells to properly repair these defects, thus preventing their transmission to the resulting daughter cells. The DNA damage checkpoint protects cells from the constant attack by exogenous as well as endogenous genotoxic agents (for example, chemicals, free radicals, ionizing radiation, by-products of intracellular metabolism or medical therapy) that induce diverse alterations in the DNA molecule. These alterations are sensed by a signalling pathway that ultimately leads to CDK inhibition and cell cycle arrest⁶. If repair is unsuccessful owing to either excessive DNA damage or genetic defects in either the checkpoint or the DNA repair machinery, cells may enter senescence or undergo apoptosis. Alternatively, accumulation of DNA alterations may result in GIN leading to cell transformation and oncogenesis³. Once the genetic material is duplicated, proper chromosome segregation is controlled by the spindle assembly checkpoint (SAC), a signalling pathway that modulates CDK1 activity and prevent defects in chromosome segregation^{4,7,8}. A defective SAC may provoke unequal inheritance of the genetic information (FIG. 1) that, if unrepaired, may facilitate tumour progression by accumulating numerical chromosomal aberrations (CIN).

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At a glance

- Cell cycle deregulation is a common feature of human cancer. Cancer cells frequently display unscheduled proliferation, genomic instability (increased DNA mutations and chromosomal aberrations) and chromosomal instability (changes in chromosome number).
- The mammalian cell cycle is controlled by a subfamily of cyclin-dependent kinases (CDKs), the activity of which is modulated by several activators (cyclins) and inhibitors (Ink4, and Cip and Kip inhibitors). The activity of cell cycle CDKs is deregulated in cancer cells owing to genetic or epigenetic changes in either CDKs, their regulators or upstream mitogenic pathways.
- Recent genetic studies indicate that CDK2, CDK4 and CDK6 are not essential for the mammalian cell cycle. Instead, they are only required for the proliferation of specific cell types. By contrast, CDK1 is essential for cell division in the embryo. Moreover, CDK1 is sufficient among the cell cycle CDKs for driving the cell cycle in all cell types, at least until mid gestation.
- Constitutive and deregulated CDK activation may contribute not only to unscheduled proliferation but also to genomic and chromosomal instability in cancer cells. The alteration of the DNA damage and mitotic checkpoints frequently results in increased CDK activity that drives tumour cell cycles.
- Emerging evidence suggests that tumour cells may also have specific requirements for individual CDKs. Therapeutic strategies based on CDK inhibition should take into consideration these specific requirements.
- For instance, CDK4 is dispensable for mammary gland development, but is required for the development of mammary gland tumours initiated by specific oncogenes such as *ErbB2*, *Hras* or *Myc* depending on cellular context.

Interphase CDKs

CDKs that contribute to several cell cycle processes during interphase. In this review, we use this term to refer to CDK2, CDK4 and CDK6. This working definition does not imply that they contribute equally to the various cell cycle events (for example, G1 progression or DNA replication) that take place during interphase.

Cell cycle checkpoint

Control mechanisms that ensure the fidelity of cell division. These checkpoints verify whether the processes at each phase of the cell cycle have been accurately completed before progression into the next phase.

DNA damage checkpoint

A signal transduction pathway induced by DNA damage that blocks cell cycle progression until DNA is properly repaired.

Spindle-assembly checkpoint

A signalling pathway that monitors the correct attachment of chromosomes to spindles. Activation of this checkpoint causes cell cycle arrest as a result of the inhibition of APC/C.

In this Review, we provide a broad perspective of how CDKs control the cell cycle and how their misregulation contributes to unscheduled proliferation, GIN and CIN during tumour development. In particular, we focus on the impact that the genetic interrogation of the role of CDKs and their direct regulators is having on our understanding of how the cell cycle is regulated in normal and tumour cells. Finally, we discuss how this information may open new avenues for cancer therapy.

CDKs and the regulation of the cell cycle

The basic regulation of the cell cycle has been well established by pioneering studies in yeasts. In these organisms, cell cycle progression is controlled by a single CDK — known as Cdc28 in *Saccharomyces cerevisiae* and Cdc2 in *Schizosaccharomyces pombe* — that binds to specific cyclins at different stages of the cycle (FIG. 1). The number of CDKs and cyclins has increased considerably during evolution (BOX 1). However, only certain CDK–cyclin complexes are thought to control cell cycle progression³. According to the ‘classical’ model for the mammalian cell cycle, specific CDK–cyclin complexes are responsible for driving the various events known to take place during interphase in a sequential and orderly fashion (FIG. 1). For instance, mitogenic signals are first sensed by expression of the D-type cyclins (D1, D2 and D3) that preferentially bind and activate CDK4 and CDK6 during G1, a phase of the cell cycle in which cells prepare to initiate DNA synthesis¹. Activation of these complexes leads to partial inactivation of the pocket proteins — RB, RBL1 (also known as p107) and RBL2 (also known as p130) — to allow expression of E-type cyclins (E1 and E2), which bind and activate CDK2 (REF. 9). CDK2–cyclin E complexes further phosphorylate these pocket proteins,

leading to their complete inactivation^{9,10}. The availability of E-cyclins during the cell cycle is tightly controlled and limited to the early stages of DNA synthesis. This observation, along with the ability of dominant-negative mutants and antibodies against CDK2 to inhibit the cell cycle in certain human tumour cell lines^{11,12}, was interpreted as evidence that CDK2–cyclin E was essential to drive the G1/S transition (reviewed in REF. 13). CDK2 is subsequently activated by cyclin A2 (cyclin A1 in germ cells) during the late stages of DNA replication to drive the transition from S phase to mitosis, a period known as the G2 phase. Finally, CDK1 is thought to be activated by A-type cyclins at the end of interphase to facilitate the onset of mitosis. Following nuclear envelope breakdown, A-type cyclins are degraded, facilitating the formation of the CDK1–cyclin B complexes responsible for driving cells through mitosis⁵. Other CDKs also have roles in the mammalian cell cycle (BOX 2).

The basic concept put forward by this model, that is, that each phase of the cycle is driven by specific CDKs, has been recently challenged by genetic studies in mice (TABLE 1). Systematic knockout of CDK loci in the mouse germline has shown that CDK2, CDK4 and CDK6 are not essential for the cell cycle of most cell types. Instead, loss of each of these CDKs results in developmental defects in highly specialized cell types. For instance, CDK4 is essential for proliferation of pancreatic β -cells and pituitary lactotrophs during postnatal development^{14,15} (TABLE 1). Likewise, loss of CDK6 causes minor defects in cells of erythroid lineage¹⁶. Mice without CDK2 do not display any detectable defects in mitotic cells^{17,18}. However, this kinase is essential for meiotic division of both male and female germ cells¹⁷. Only elimination of the mitotic kinase CDK1 causes cell cycle arrest, preventing embryos from developing beyond the two-cell stage¹⁹ (M.M. and M.B., unpublished observations).

The absence of major cell cycle defects in cells lacking individual interphase CDKs is not simply due to compensatory activities among these CDKs. Concomitant loss of multiple CDKs enhances the scope of these developmental defects but does not result in a general disturbance of the cell cycle in most cell types. For instance, ablation of *Cdk4* and *Cdk6* results in impaired proliferation of haematopoietic precursors, leading to late embryonic death¹⁶ (FIG. 2). However, no defects in G1 phase or cell cycle re-entry in cells other than those of haematopoietic lineage were observed¹⁶. A major compensatory effect from the third interphase CDK, CDK2, has also been ruled out. Mice lacking CDK2 and CDK6 reach adulthood and do not display any defects except those observed in the single mutant strains¹⁶. Likewise, mice lacking CDK2 and CDK4 complete embryonic development and do not show cell cycle defects except in embryonic cardiomyocytes, another highly specialized cell type^{20,21} (FIG. 2). These observations are not unique to embryonic cells, as adult mice lacking CDK2 and CDK4 do not display obvious defects²⁰. Indeed, these mice recover normally after severe hepatectomy, indicating that adult hepatocytes proliferate normally without CDK2 and CDK4 kinases²⁰. Interestingly, there is no promiscuity between interphase and mitotic CDKs.

The fact that embryos lacking CDK1 do not divide in spite of carrying the full complement of interphase CDKs illustrates that these CDKs cannot compensate for the absence of CDK1 (REF. 19). Moreover, replacement of *Cdk1* by *Cdk2* using homologous recombination also results in early embryonic lethality²², indicating that CDK1 cannot be compensated for by CDK2, even when expressed from the *Cdk1* locus.

Ablation of the genes encoding individual D-type cyclins leads to specific developmental defects, most probably owing to their differential patterns of expression²³. Concomitant ablation of the three D-type cyclins results in embryonic lethality owing to haematopoietic defects similar to those observed in mice lacking CDK4 and CDK6, suggesting that the D-type cyclins are the functional activators of these G1 kinases^{16,24}. Ablation of the genes encoding cyclin E1 and cyclin E2 also results in embryonic lethality, in this case owing to specific defects in the endo-reduplication of trophoblast cells^{25,26}. However, these two proteins are not required for proliferation of most embryonic cells or for full development of the embryo when the placental defect is rescued²⁵. Intriguingly, CDK2 deficiency does not cause placental defects, suggesting that E-type cyclins have CDK2-independent roles, possibly in controlling DNA replication²⁷. Interestingly, expression of cyclin E1 within the cyclin D1 locus rescues most phenotypes observed in cyclin D1-null mice, thus revealing an interesting functional overlap between different classes of cyclins²⁸. Knockout of cyclin A2 leads to early embryonic lethality²⁹, suggesting that the main role of this cyclin is to activate the mitotic CDK, CDK1. Finally, knockout of *cyclin B1* also results in early embryonic death, an expected observation considering the key role of this cyclin in mitosis³⁰.

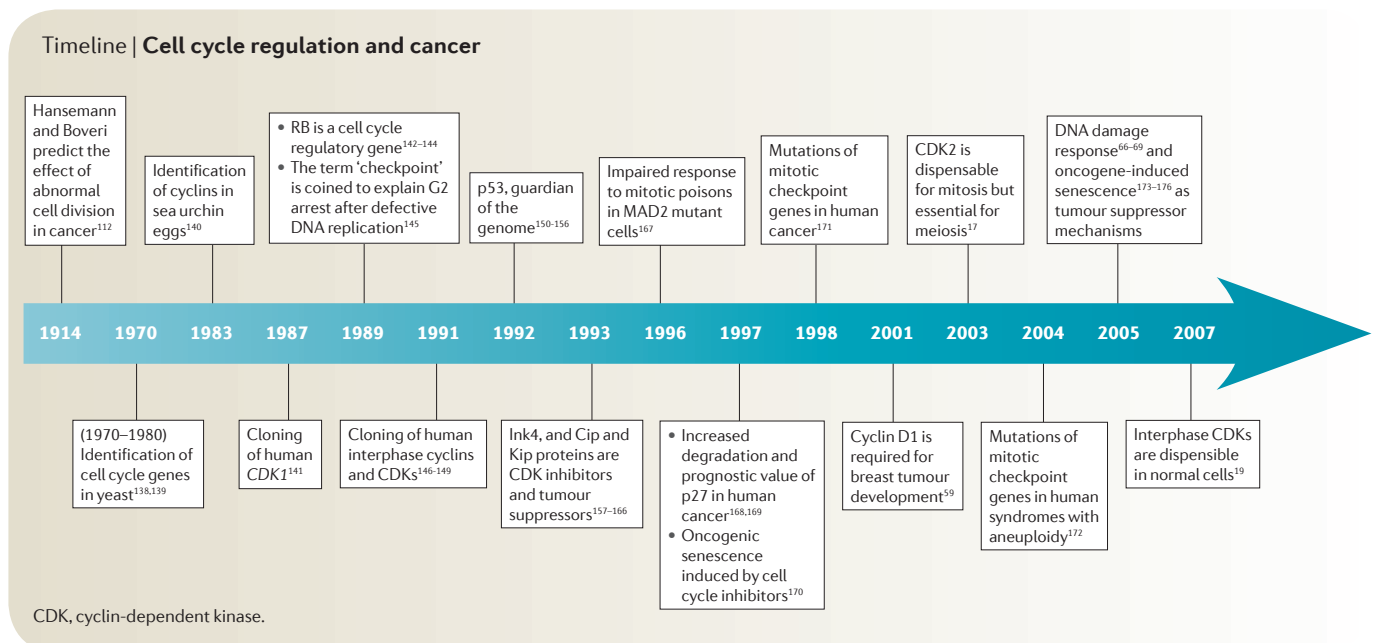
Arguably, the most striking result derived from genetic interrogation of the mammalian cell cycle came from the observation that mouse embryos develop normally

until mid gestation without all interphase CDKs¹⁹ (FIG. 2). Although these embryos fail to thrive owing to defective haematopoiesis, none of the other cells display obvious cell cycle defects, except for cardiomyocytes, which appear in decreased numbers. As early development is the period of most active cell division and when organogenesis takes place, these observations imply that CDK1 is sufficient to drive cell division in most cellular lineages. These observations do not imply that interphase CDKs do not contribute to normal cell cycles even in those cells in which they are dispensable. However, the fact that CDK1 alone can drive the cell cycle in most mammalian cells suggest that the mammalian cell cycle is not conceptually too different from that of yeast (FIG. 1).

Why have eukaryotes evolved an increasing number of CDKs? The most plausible explanation is that multicellular organisms require additional levels of regulation to control proliferation of a wide repertoire of cell types. At least, some of these controls may involve phosphorylation of specific substrates in order to allow specialized cells to proceed through the cell cycle. However, this hypothesis needs to be validated by experimental evidence. Regardless of the function that interphase CDKs may have within the normal cell cycle, these observations have raised an important issue: do tumour cells retain the same CDK requirements as the normal cells from which they originate or do they acquire specific needs for CDK activity during tumour development? The original observations of Harlow and co-workers¹¹ showing that certain human tumour cells require CDK2, an interphase CDK dispensable for normal homeostasis^{17,18}, suggested that the latter might be the case (see below).

CDKs, CDK inhibitors and cancer

Homeostasis within adult tissues is characterized by a quiescent stem cell pool intermittently yielding



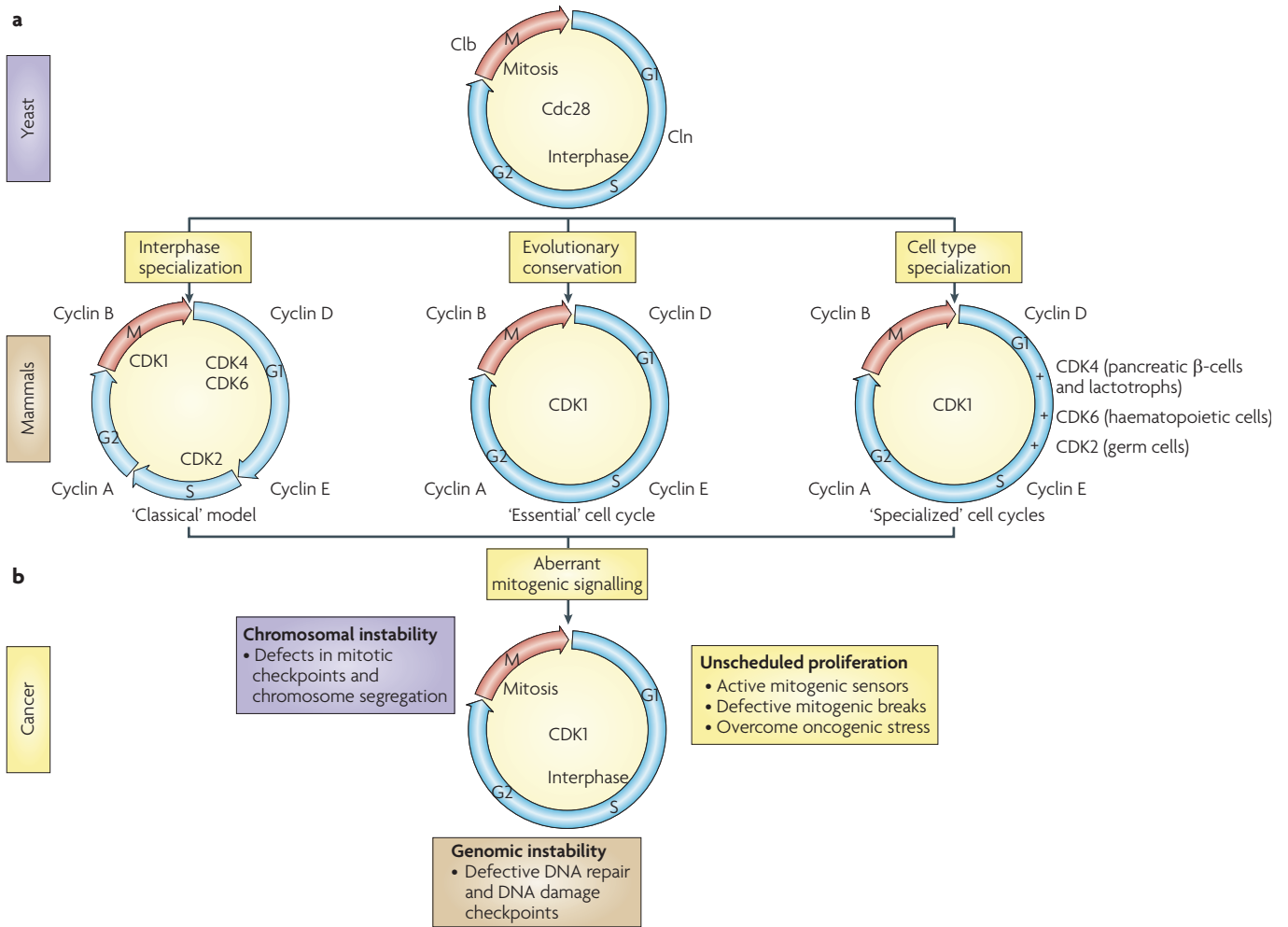


Figure 1 | Comparison of the *Saccharomyces cerevisiae* cell cycle with the mammalian cell cycle, highlighting the major defects implicated in human cancer. **a** | The diagram depicts the *S. cerevisiae* cell cycle and three models of the mammalian cell cycle. In the currently accepted model based on biochemical evidence ('classical' model), each of the main events that take place during interphase (G₁, S and G₂) is driven by unique cyclin-dependent kinases (CDKs) bound to specific cyclins. The 'essential' cell cycle is based on genetic evidence indicating that CDK1 is sufficient to drive proliferation of all cell types up to mid gestation¹⁹ as well as during adult liver regeneration. The 'specialized' cell cycles are based on the unique requirements of specialized cell types for specific CDKs as indicated (TABLE 1). **b** | Unscheduled cell proliferation requires aberrant mitogenic signalling driven by either excessive exogenous signalling (for example, growth factors and nutrients) or endogenous oncogenic mutations. Other mutations affecting mitogenic breaks (for example, tumour suppressors and negative regulators) that decrease the threshold for mitogenic signalling also contribute to unscheduled proliferation. Oncogene-induced cell cycling provokes DNA replication stress that is sensed by the DNA replication checkpoints. Failure in this control mechanism results in an increased mutation rate and ultimately may lead to genomic instability. During mitosis, defects within the spindle assembly checkpoint induce deregulation of CDK1 activity that may result in abnormal chromosome segregation. All these defects converge in deregulation of CDK activity, eventually leading to tumour development.

daughter progenitor cells with high proliferative capacity. Quiescence is necessary to prevent premature exhaustion of the repopulating ability of adult stem cells over the lifetime of individuals. Recent evidence indicates that specific CDK–cyclin complexes, modulated by CDK inhibitors (CKIs), may be responsible for maintenance of the quiescent state in different stem cell populations. Whereas CDK downregulation may result in defective homeostasis in specific tissues, hyperactivation of CDKs may favour tumour development by inducing unscheduled cell division in either stem or, possibly, progenitor cells.

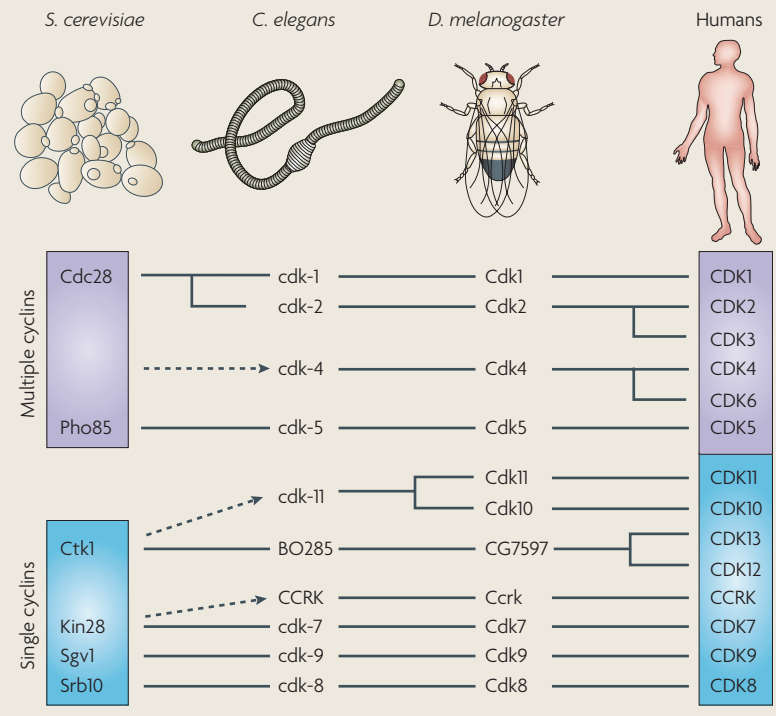
Control of stem or progenitor cell proliferation. CDK activity is regulated by two families of inhibitors: INK4 proteins, including *INK4A*, *INK4B*, *INK4C* and *INK4D*, and the Cip and Kip family, composed of *p21*, *p27* and *p57* (REF. 5). These cell cycle inhibitors have been shown to block proliferation of adult stem cells in multiple tissue types. For instance, *p21* and *p27* may control self-renewal of neural, intestinal and haematopoietic progenitors^{31–36}. Knock-in mice expressing a mutant *p27* protein unable to bind CDK–cyclin complexes display expanded stem and progenitor cell populations as well as a wide range

Box 1 | Evolution of cell cycle control: from yeast to humans

The *Saccharomyces cerevisiae* genome encodes about 23 cyclins, which regulate six proline-directed serine/threonine protein kinases: Cdc28 (cell division control 28), Pho85, Kin28, Srb10 (also known as cyclin-dependent kinase 8 (Cdk8) and Ccn3), Sgv1 (also known as Bur1) and Ctk1. Two of these kinases, Cdc28 and Pho85, are activated by large cyclin families, whereas the other kinases associate with a single dedicated cyclin. Cdc28 and its associated cyclins are essential for driving the cell cycle. The multifunctional kinase Pho85 regulates G1 progression, cell polarity and the actin cytoskeleton, transcription, phosphate and glycogen metabolism, and senses changes in the environment¹¹⁵. The four yeast CDKs that associate with a single cyclin have close ties with the transcriptional machinery and probably control gene expression through regulation of the RNA polymerase II holoenzyme and transcription factors.

In humans, the CDK family is composed of 13 members (BOX 2) that interact with at least 29 cyclins or cyclin-related proteins⁵. An additional family of five proteins (known as Ringo or Speedy) with structural but not sequence homology to cyclins, have been found in vertebrates but not in *S. cerevisiae*, *Caenorhabditis elegans* or *Drosophila melanogaster*¹¹⁶. Whereas some transcriptional CDKs such as CDK7, CDK8 and CDK9, have not diverged much from their yeast orthologues, cell cycle CDKs have evolved in metazoans to generate new CDK subfamilies, such as CDK4 and CDK6 (see the figure).

In *S. cerevisiae*, nine different cyclins (Cln and Clb subgroups) bind and activate Cdc28. Progression through G1 requires Cdc28 and at least one of the G1 cyclins (Cln1, Cln2 and Cln3), whereas efficient DNA replication requires Cdc28 and the early expressed B-type cyclins (Clb5 and Clb6). Functional complexes of Cdc28 and mitotic cyclins (Clb1, Clb2, Clb3 and Clb4) are essential for mitotic events¹¹⁷. Pho85, on the other hand, associates with 10 cyclins (Pcl subgroup) that function in nutrient sensing. At least four of these Pcls (Pcl1, Pcl2, Pcl7 and Pcl9) exhibit cell cycle periodic expression from early G1 to S phase. A specific function for Pho85 in sensing mitogenic signals for G1 progression has been suggested¹¹⁵. The increased number of cyclins in the mammalian genome has resulted in a large variety of CDK–cyclin complexes^{5,116}. However, only ten cyclins (three D-type, two E-type, two A-type and 3 B-type cyclins) are known to be directly involved in driving the mammalian cell cycle.



of tumours³⁷. Similarly, INK4A, INK4B and INK4C are involved in modulating the self-renewal of haematopoietic, brain, lung and pancreatic stem and progenitor cells^{38–44}. In turn, the expression levels of CKIs are

controlled by crucial pathways that modulate stem cell functionality. For instance, activation of Notch signalling blocks transforming growth factor- β (TGF β)-dependent upregulation of INK4B, INK4A, p21 and p27 by modulating the binding of phospho-SMAD3 to their respective promoters. On the other hand, inhibition of Notch signalling induces expression of these CKIs⁴⁵. CKI levels are also crucial in limiting the self-renewal potential of stem cells, as attenuation of the TGF β –SMAD3 pathway by RNA interference decreases the protein levels of INK4B and p21 and restores regeneration of muscle cells⁴⁵.

These results indicate that the loss of CKIs may initially expand the stem cell population, possibly contributing to the development of specific tumours. However, sustained loss may lead to exhaustion of self-renewal potential and a decrease in stem cell number or functionality under stress conditions. The fact that CDK ablation results in defective self-renewal ability indicates that the effects of CKIs in progenitor cells are mediated by their CDK inhibitory function. Thus, CDK4 is repressed in quiescent skin stem cells but induces precocious follicular growth when this repression is relieved⁴⁶. Adult CDK2-deficient neural progenitors display decreased self-renewal capacity and enhanced differentiation⁴⁷. These defects are enhanced after suppressing CDK4 expression in CDK2-deficient cells, suggesting that several CDKs may cooperate in the self-renewal ability of these progenitors⁴⁷.

Information from these mouse models suggests that interphase CDKs such as CDK2 or CDK4 are modulated by CKIs to control the proliferative potential and self-renewal abilities of normal stem or progenitor cells. In human tumours, inappropriate regulation of CDKs in putative cancer stem cells may induce tumorigenesis. Short treatments with specific CDK inhibitors may lead to transient quiescence of cancer stem cells that can resume their proliferative activity after treatment. Definition of the requirements of interphase CDKs in normal and cancer stem cells may help in the development of novel therapeutic strategies specifically targeted to cancer stem cells.

Deregulation of interphase CDKs in tumours. The mutations in CDKs and their regulators that contribute to human cancer have been well defined. Deregulation of CDK4 and CDK6 activities have been implicated in a wide variety of tumours^{1,5,48}. CDK4 is altered in a small set of melanoma patients by a missense mutation (Arg24Cys) that blocks binding of INK4 inhibitors. CDK6 is overexpressed in some leukaemias as a consequence of nearby translocations. *CDK4* and *CDK6* are also amplified or overexpressed in several malignancies (including sarcoma, glioma, breast tumours, lymphoma and melanoma). However, the causal role of these alterations in tumour development is difficult to assess, for example, *CDK4* is co-amplified with *MDM2* in most of these tumours⁵. Misregulation of D-type cyclins and INK4 inhibitors is a common feature of most tumour types^{1,48}, suggesting that CDK4 and CDK6 kinases are hyperactive in human cancer with preference for CDK6 in mesenchymal tumours (leukaemias and sarcomas), and CDK4 in epithelial malignancies (in endocrine tissues and mucosae) and

in some sarcomas. *CDK2* has not been found mutated in human cancer. However, E-type cyclins are often over-expressed in human tumours, and expression of the p21 and p27 inhibitors is frequently silenced during tumour development¹. These observations suggest a potential involvement of *CDK2* in human cancer.

Experimental deregulation of the cell cycle in mice also leads to tumour development. For instance, constitutive activation of *CDK4* in a knock-in strain that carries the miscoding mutation (Arg24Cys) found in melanoma patients results in endocrine neoplasias (insulinomas, and Leydig cell and pituitary tumours), epithelial hyperplasias (of the liver, gut and breast) and sarcomas, albeit after long latencies^{49,50}. Interestingly, these mice do not develop melanoma unless insulted with a skin carcinogen⁵¹. So far there are no models for *CDK6*- or *CDK2*-induced tumorigenesis. Ablation of either p21 or p27 in the germ line of mice results in tumour development^{1,5}. Interestingly, genetic experiments have indicated that *CDK2* does not have a significant role in tumours lacking these inhibitors^{52,53}. As p21 and p27 also inhibit *CDK1*, it is possible that deregulation of *CDK1* activity might be responsible for tumour development in those malignancies lacking p21 or p27 expression.

Do tumour cells require specific CDKs for proliferation?

As summarized above, some CDKs are only essential for the proliferation of specialized normal cells. As *CDK4*

is essential for the proliferation of mouse pancreatic β -cells, would inhibition of *CDK4* have therapeutic activity against insulinomas? Likewise, would concomitant inhibition of *CDK4* and *CDK6* stop proliferation of haematopoietic malignancies? Considering the wider picture, is it possible that tumour cells, depending on their developmental origin, require particular interphase CDKs for proliferation? Alternatively, do they acquire such dependence on the basis of their pathogenic pattern of mutations? Experimental evidence suggests that some human tumour cell lines display a selective dependence on interphase CDKs. For instance, whereas colon carcinoma cell lines efficiently proliferate in the absence of *CDK2*, downregulation or inhibition of this kinase in cell lines derived from glioblastomas and osteosarcomas prevents their proliferation^{11,54}. As *CDK2*, at least in mice, is not required for proliferation of cells of brain or connective tissue, the requirement of these tumour cells for *CDK2* must be acquired during the neoplastic process.

Similar observations are now emerging from more sophisticated studies using gene-targeted mouse tumour models. *Cdk4*-null mice, unlike their wild type counterparts, do not develop skin tumours induced by *Myc*⁵⁵. Similarly, *Cdk4*-deficient mice are resistant to mammary tumours expressing *ErbB2* and *Hras* under the control of the mouse mammary tumour virus promoter^{56,57}. However, expression of *CDK4* does not appear to be essential for mammary gland development. Similarly, mice lacking cyclin D1 or expressing a cyclin D1 mutant that does not activate *CDK4* are also resistant to breast tumours induced by ERBB2 (REFS 58,59). However, lack of cyclin D1 has no effect on breast tumour development induced by *Myc* or *Wnt1* (REF 59). These observations indicate that active *CDK4*-cyclin D1 complexes are required for skin or breast tumour development, depending on the nature of the oncogenic insult. Thus, *CDK4* inhibition by small molecules may have therapeutic value in treating ERBB2-positive breast tumours⁶⁰. We have observed similar results when *CDK4*, but not *CDK6*, is ablated in non-small-cell lung tumours induced by expression of endogenous *Kras* (M.M. and M.B., unpublished observations). That *CDK* inhibition could have therapeutic value in the treatment of selective malignancies based on their acquired and/or innate dependency of interphase CDKs is an interesting possibility that deserves to be explored.

GIN and the DNA damage response

CDK activity is also regulated by checkpoints that prevent cell cycle progression until cells are assured that the previous phase has been successfully accomplished. Mutations that affect checkpoint control result in deregulation of *CDK* activity, ultimately leading to malignant transformation. The best illustrated relationship between loss of a cell cycle checkpoint, *CDK* activity and cancer is the DNA damage checkpoint^{3,6}. Replicated DNA is proofread to ensure the accuracy of the replicative process. In addition, cells are exposed to exogenous and endogenous insults that can induce small mutations as well as gross chromosomal rearrangements^{6,61}.

Box 2 | Contributions to cell cycle control by other mammalian CDKs

In addition to the interphase cyclin-dependent kinases (*CDKs*; *CDK2*, *CDK4* and *CDK6*) and the mitotic *CDK* (*CDK1*), other *CDK* family members have been implicated in the regulation of the cell cycle.

Human *CDK3* has been reported to partner with cyclin C to act during interphase¹¹⁸. Unfortunately, *CDK3* is inactive in most strains of laboratory mice¹¹⁹ and has received relatively little attention.

CDK5 is primarily active in post-mitotic neurons and phosphorylates several cytoskeletal proteins⁵. Recent data suggest that *CDK5* is essential for neuronal cell cycle arrest and differentiation and it may be involved in apoptotic cell death in neuronal diseases^{120,121}.

CDK7 partners with cyclin H to phosphorylate and activate *CDKs*, mainly *CDK2* and *CDK1* (REF. 108). The *CDK7*-cyclin H kinase also controls transcription by associating with TFIIF and phosphorylating the carboxy-terminal domain of the largest subunit of RNA polymerase II¹²². As previously suggested¹²³, the dual role of *CDKs* in cell cycle control and transcription may facilitate the coupling of these processes during cell cycle progression.

CDK8 and *CDK9*, on the other hand, participate in transcription by phosphorylating other RNA polymerase components⁵. Recently, *CDK8* has been shown to promote colon cancer by a mechanism involving gene amplification^{124,125}. Increased *CDK8* kinase activity is necessary for the repression of apoptotic E2F1 targets as well as for expression of several β -catenin transcriptional targets.

CDK10 and *CDK11* display distinct functions during the G2/M transition, in addition to their roles in regulating transcription⁵. Interestingly, *CDK11* is essential for mouse development¹⁰⁹ and has been shown to participate in centrosome maturation, spindle formation, sister chromatid cohesion and cytokinesis^{126–129}. These two mammalian *CDKs* do not have a clear orthologue in yeast although they are more similar to Ctk1 than to the other yeast *CDKs* (BOX 1). As Ctk1 is mainly involved in transcriptional and translational control and modulating chromatin structure¹³⁰, the centrosomal and mitotic functions of *CDK11* may have emerged later in evolution.

CDK12 (previously known as Crkr) and *CDK13* (*CDC2L5*) are a subfamily of *CDKs* that bind L-type cyclins and participate in alternative splicing regulation^{131,132}.

The mammalian genome also contains several other *CDK*-like proteins for which the cyclin partner and functions are unknown at present⁵.

Table 1 | Representative mouse models carrying gene-targeted CDK alleles*

Kinase	Genotype [§]	Phenotype	Refs
Loss-of-function strains			
CDK1	<i>Cdk1</i> ^{mut/mut}	Deficiency in CDK1 results in embryonic lethality in the first cell divisions	19
CDK2	<i>Cdk2</i> ^{-/-}	Sterility due to defective meiosis; no effect on mitotic cells	17,18
CDK4	<i>Cdk4</i> ^{-/-}	Diabetes and defective postnatal proliferation of endocrine cells such as pancreatic β -cells or pituitary hormone-producing cells	14,15, 133–136
CDK6	<i>Cdk6</i> ^{-/-}	Slight anaemia and defective proliferation of some haematopoietic cells	16
CDK11	<i>Cdk11</i> ^{-/-}	Embryonic lethality in peri-implantation embryos accompanied by mitotic aberrations	109
CDK2; CDK4; CDK6	<i>Cdk2</i> ^{-/-} ; <i>Cdk4</i> ^{-/-} ; <i>Cdk6</i> ^{-/-}	Deficiency in all these interphase CDKs provokes embryonic lethality by mid-gestation due to haematopoietic defects	19
Target validation strains			
CDK2	<i>Cdk2</i> ^{-/-} ; <i>Cdkn1b</i> ^{-/-}	Develop tumours with similar incidence and latency to those in <i>Cdkn1b</i> -deficient mice, suggesting the function of p27 (encoded by <i>Cdkn1b</i>) is independent of CDK2	52,53
CDK4	<i>Cdk4</i> ^{-/-} ; K5-Myc	Resistant to Myc-induced skin tumours	55
CDK4	<i>Cdk4</i> ^{-/-} ; MMTV- <i>ErbB2</i>	Resistant to <i>ErbB2</i> -induced breast tumours	56,57
CDK4; CDK6	<i>Ccnd1</i> ^{K112E/K112E} ; MMTV- <i>ErbB2</i>	Resistant to <i>ErbB2</i> -induced breast tumours	58
Gain-of-function strains			
CDK4	<i>Cdk4</i> ^{R24C/R24C}	Mice expressing an endogenous Ink4-insensitive CDK4 ^{R24C} mutant develop a variety of tumour types with complete penetrance	49,51
CDK4	<i>Cdk4</i> ^{R24C/R24C} ; <i>Cdkn1b</i> ^{-/-}	Mice develop aggressive pituitary tumours with short latency (8–10 weeks)	49,137

*These mouse strains are also deficient in cyclin-dependent kinase 3 (CDK3) owing to a naturally occurring mutation present in most inbred strains that results in inactivation of CDK3 owing to premature termination of its coding sequence¹¹⁹. [§]The *Cdk1*^{mut} allele was generated by insertion of a gene trap vector. *Ccnd1*^{K112E} is a knock-in allele in which Lys112 has been replaced by Glu to prevent binding of cyclin D1 to CDK4 and CDK6. *Cdk4*^{R24C} is a knock-in allele in which Arg24 has been replaced by Cys to prevent binding of Ink4 inhibitors. All the other alleles (indicated by a minus) were generated by classical knockout strategies. Finally, MMTV-*ErbB2* is a transgene in which the *ErbB2* receptor tyrosine kinase coding sequence is driven by the mouse mammary tumour virus promoter. K5-Myc is a transgene in which the *Myc* oncogene is driven by the keratin 5 promoter.

The molecular pathways that prevent cells from proliferating until damaged DNA has been properly repaired have been extensively reviewed^{3,6,62}. Briefly, they involve the sensor kinases ataxia-telangiectasia mutated (*ATM*) and ataxia-telangiectasia and Rad3-related (*ATR*), and the checkpoint kinases *CHK1* and *CHK2*. Activation of these pathways in response to DNA damage results in increased levels of the CDK inhibitor p21 or inhibition of CDK activators such as the Cdc25 phosphatases⁶. These mechanisms prevent G1/S or G2/M transitions in the presence of DNA damage, mostly by inhibiting CDK activity. Mutations in DNA damage checkpoint proteins predispose carriers to specific syndromes, such as ataxia-telangiectasia (*ATM* mutations), Seckel (*ATR* mutations) and Li-Fraumeni (*CHK2* mutations) syndromes, all of which display increased susceptibility to cancer^{62–65}. In addition, recent studies have proposed that the DNA damage response may act as an anticancer barrier^{66–69}. Proliferative forces such as activation of the Ras, MYC or E2f signalling pathways induce DNA damage-responses. It is therefore not surprising

that human tumour cells often show constitutive activation of DNA damage signalling pathways, represented by activated ATM, CHK1 or CHK2 kinases, phosphorylated histone H2AX (γ H2AX) and p53, all of which are markers of a DNA damage response⁷⁰. Moreover, activation of the DNA damage response is at its maximum level in the early, pre-invasive stages of human tumours and correlates with the presence of senescence markers⁷⁰. These markers precede the deregulation of the DNA damage response pathway by specific mutations in its regulators, mainly the ATM-CHK2-p53 pathway. Under these conditions, CDKs become hyperactive owing to the reduced expression of their CDK inhibitor p21 in the absence of p53, its primary transcriptional activator. Likewise, CDKs also become hyperactive by constitutive activation of Cdc25 phosphatases, which eliminate inhibitory phosphorylation sites on CDK molecules. These alterations, taken together, result in cell cycle progression in the presence of damaged DNA, leading to increased GIN (FIG. 1).

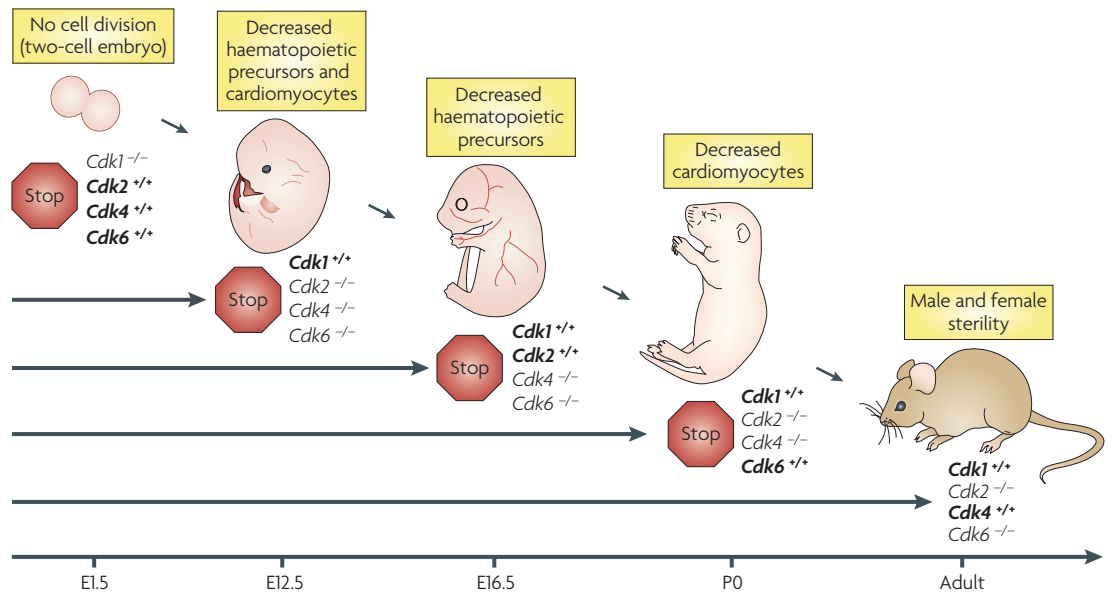


Figure 2 | Genetic interrogation of the roles of cyclin-dependent kinases (CDKs) in the mammalian cell cycle. The genotypes of the various CDK mutant strains used in this analysis are indicated. Wild-type CDKs are indicated in bold font. Ablated CDKs appear in normal font. The arrows indicate the extent to which each of the mouse strains develops with the indicated CDK content. Stop signs indicate the developmental stage at which the strains are no longer viable. The main defects responsible for loss of viability are also indicated. Briefly, mice expressing all interphase CDKs but not CDK1 do not progress beyond the two-cell embryo stage. Mice expressing CDK1 but no interphase CDKs, progress up to mid gestation (embryonic day (E)12.5–E13.5). Mice lacking CDK4 and CDK6 develop until late embryonic development (E16.5–E17.5), whereas those lacking CDK4 and CDK2 die at birth. Finally, mice lacking CDK6 and CDK2 develop to adulthood and have a normal life span. Except for embryos lacking CDK1, none of the mutant embryos or mice display cell cycle defects except in those cell types indicated in the yellow boxes. For specific information regarding the phenotypes of single mutant strains see TABLE 1. P, postnatal day.

In addition to the role of CDKs as end-point effectors of the DNA damage checkpoint, there is increasing evidence that the catalytic activities of CDKs may have specific roles during DNA repair⁷¹. The yeast Cdk1 seems to have a key role in selecting between the two main mechanisms used to repair double-strand breaks: non-homologous end-joining, which is mostly active during G1, and homologous recombination, which is active during S phase and G2 (REF. 72). In humans, CDK1 and CDK2 phosphorylate the tumour suppressor *BRCA2* to modulate its interaction with *RAD51*. This interaction stimulates homologous recombination-dependent repair during S phase and G2 (REF. 73). To what extent the hyperactivation of CDKs may participate in GIN by directly deregulating DNA repair is unknown at present.

CDK1, mitosis and chromosomal instability

During mitosis, the replicated genetic material and centrosomes are equally distributed between the two daughter cells. CDK1, in complex with A- or B-type cyclins, is one of the master regulators of mitosis as it controls the centrosome cycle as well as mitotic onset. Active CDK1–cyclin complexes phosphorylate more than 70 substrates during G2 and early mitosis to trigger centrosome separation, Golgi dynamics, nuclear envelope breakdown and chromosome condensation, among other processes⁵. Once chromosomes are condensed and aligned at the metaphase plate, CDK1 activity is switched off to allow sister chromatid separation through activation of

separase (also known as *separin*)⁸, a protease that cleaves the cohesin subunit *SCC1* (also known as *RAD21*), eliminating chromatid cohesion (FIG. 3). Inactivation of CDK1 is also required for chromosome decondensation, re-formation of the nuclear envelope and cytokinesis⁷⁴. Inactivation of CDK1 is accompanied by activation of Cdc14 proline-directed phosphatases, and possibly other phosphatases such as PP1 or PP2, to remove CDK1-dependent phosphates from mitotic substrates⁷⁵.

The spindle assembly checkpoint (SAC). SAC is a signalling pathway that ensures proper segregation of sister chromatids by inhibiting the metaphase–anaphase transition until all chromosomes are bipolarly attached to the mitotic spindle⁸. The molecular pathways that regulate SAC function have been extensively reviewed^{4,8,76,77}. Briefly, chromosome segregation is mediated by the anaphase-promoting complex/cyclosome (APC/C), an E3 ubiquitin ligase responsible for targeting mitotic cyclins, among other mitotic regulators, for degradation^{76,78}. Target selection is mediated by the APC/C cofactors, *CDC20* or *CDH1* (also known as FZR1). *CDC20* is the key APC/C cofactor of this pathway: it targets *securin* (encoded by *PTTG1*) and cyclin B for degradation once attachment is completed (FIG. 3). Cyclin B is an obligatory target for APC/C to switch off CDK1 activity during anaphase. If CDK1 activity is recovered after chromosome segregation, chromosomes re-condense and cells are not able to exit from mitosis⁷⁴. *CDC20* is also crucial

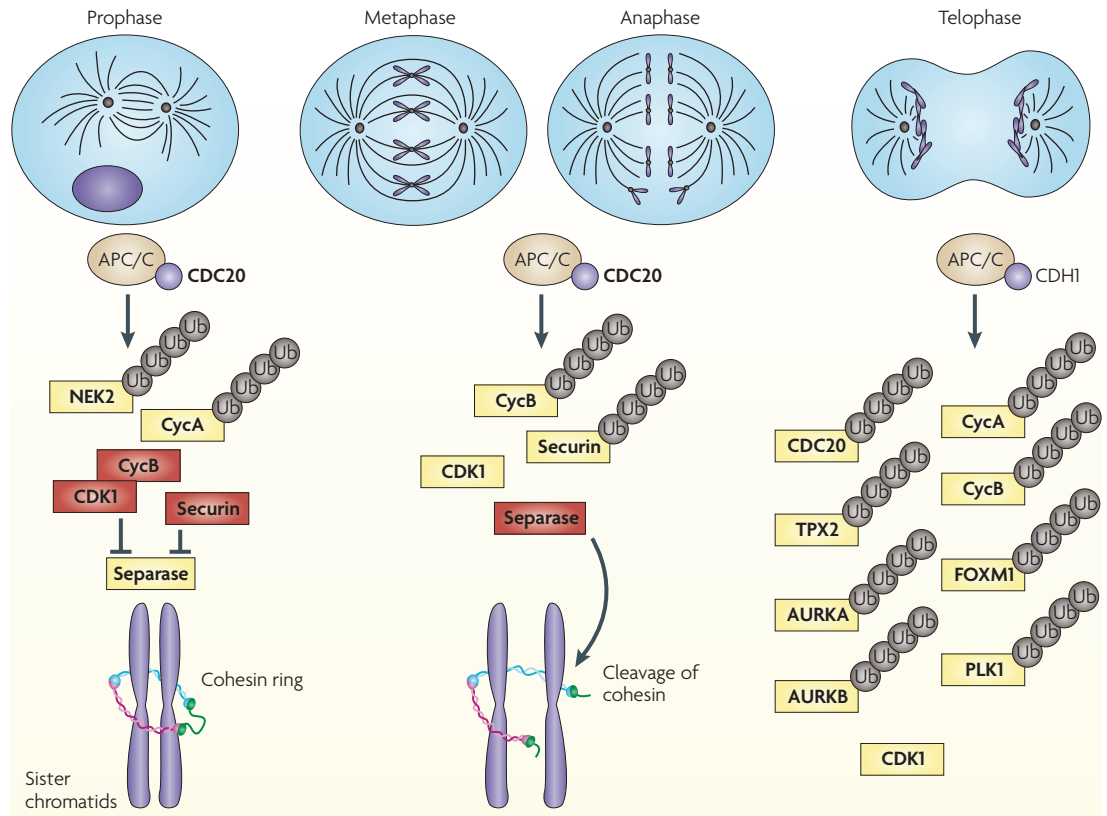


Figure 3 | An overview of key molecules involved in mitotic progression and chromosomal instability. The anaphase-promoting complex/cyclosome (APC/C)–CDC20 (cell division control 20) complex targets cyclin A (CycA) and NEK2 for degradation by ubiquitylation in a spindle assembly checkpoint (SAC)-independent manner. In the presence of unaligned chromosomes, separase is kept inactive by securin and CDK1–cyclin B. Under these conditions, sister chromatids are held together by cohesins. After complete bipolar attachment of chromosomes to the mitotic spindle, cyclin B and securin are also ubiquitylated by APC/C–CDC20, but in a SAC-dependent manner. Ubiquitin-dependent degradation of these proteins inhibits cyclin-dependent kinase 1 (CDK1) leading to the activation of separase, which in turn cleaves cohesins and releases sister chromatids, hence facilitating the metaphase-to-anaphase transition. APC/C–CDH1 also targets cyclin A and cyclin B to keep low levels of CDK1 activity during mitotic exit and the following G1 phase. Other APC/C–CDH1 substrates involved in mitotic progression include CDC20, TPX2, forkhead box protein M1 (FOXM1), aurora kinase A (AURKA), AURKB and PLK1. Additional APC/C–CDH1 targets involved in the control of DNA replication, such as SKP2, CDC6 or geminin, are omitted for clarity. Tumours with chromosomal instability are characterized by molecular signatures in which most of these molecules (in bold) are overexpressed⁸¹. Active proteins are represented by red boxes.

for targeting cyclin A and **NEK2** for degradation (FIG. 3), although this activity is independent of SAC^{79,80}. After anaphase, APC/C function is maintained by CDH1, a cofactor that mediates the ubiquitylation of a number of mitotic (cyclin A, cyclin B, **TPX2**, aurora kinases A and B, **PLK1** and CDC20 itself) and DNA replication (**CDC6**, **geminin** and the F-box protein **SKP2**) proteins, preventing CDK1 activation during exit from mitosis and G1 phase of the following cell cycle⁷⁶ (FIG. 3).

Centrosomal and chromosomal defects in CIN tumours. Detailed analysis of genes deregulated in chromosomally unstable tumours indicates a high enrichment for centrosomal and mitotic genes in their expression signatures^{7,81} (FIG. 3). These signatures include overexpression of CDK1 and some of its regulators such as cyclin B1, cyclin B2, **CKS1** and **CKS2**. The CIN signature includes additional components of the centrosome and chromosome segregation machinery, such as **NEK2**, aurora

kinase A, aurora kinase B, **CDC20**, **CDCA8** (also known as borealin), **CENPE**, separase (encoded by **ESPL1**), securin, **TTK** or **MAD2L1** (REF. 81).

Aberrations in centrosome number or size are frequently observed in tumour cells⁸². Centrosomes have a crucial role in the formation of bipolar mitotic spindles, which are essential for accurate chromosome segregation. CIN tumours frequently overexpress centrosome proteins such as CDK1, as well as cyclins E, A and B, in addition to CDC25 (also known as **RASGRF1**), **NEK2**, aurora kinase A, **PLK1** or **TPX2** (REFS 81,82). CDK2–cyclin E, CDK2–cyclin A and perhaps CDK1 activity is thought to induce centrosome amplification in p53-defective cells^{82–85}. Centrosome amplification may lead to the formation of aberrant mitotic spindles with multiple spindle poles that result in abnormal cell divisions and aneuploidy (CIN).

SAC regulators are also frequently altered in CIN tumours^{4,7}. Whereas some of them are frequently overexpressed, others such as **BUB1**, **BUBR1**, **MAD1** and

MAD2L1 display loss-of-function mutations^{4,7}. More recently, sequencing of human cancer genomes has revealed mutations in mitotic kinases such as aurora and Polo kinases, as well as in members of the Nek, Lats and Tlk families⁸⁶. Whereas there are a significant number of GIN syndromes caused by mutations in DNA damage response^{62–65}, only a few CIN syndromes exist in humans, such as *mosaic variegated aneuploidy* or the closely related *premature chromatid separation* syndrome. These tumour susceptibility syndromes result from inactivating mutations in *BUBR1* (REFS 4,87). The limited number of CIN syndromes is probably due to the fact that chromosomally unstable embryos frequently die in the first stages of embryonic development^{4,88}. A limited number of gene-targeted mouse models have also illustrated a connection between mitotic regulation, CIN and cancer. Partial inactivation of mouse *Bub1* or *Bub1* results in CIN and increased tumour susceptibility *in vivo*^{89–92}. Both MAD2L1-deficient and MAD2L1-overexpressing mice also develop chromosomally unstable tumours^{93,94}, suggesting that unbalanced SAC regulation favours CIN. Whether CIN is sufficient for initiating tumour development or can only contribute to tumour progression remains controversial^{95,96}.

Several proteins deregulated in the CIN signature are APC/C targets that modulate CDK activity (FIG. 3). The relevance of APC/C in CDK regulation has been further illustrated by eliminating the APC/C cofactor CDH1 in mice. In the absence of CDH1, cells express high levels of A-type and B-type cyclins, leading to higher CDK activity, which ultimately provokes a shortened G1 phase, abnormal DNA replication and GIN^{97–99}. *In vivo*, partial inactivation of murine *Cdh1* results in the development of epithelial tumours, suggesting that *Cdh1* is haploinsufficient for tumour suppression⁹⁸. The relevance of CDH1 in tumour formation is highlighted by the fact that most APC/C–CDH1 substrates, including aurora kinase A, aurora kinase B, CDC20, PLK1, CDC6, securin and SKP2, are oncogenic molecules often upregulated in human CIN tumours (FIG. 3). These tumours also overexpress cyclin A and cyclin B as well as the CDK1 regulatory proteins CKS1 and CKS2, possibly resulting in abnormal regulation of CDK1. Whether these tumour cells display increased sensitivity to CDK1 inhibitors remains to be tested.

Therapeutic implications

The central part that CDKs and other kinases play in controlling the mammalian cell cycle and its checkpoints raises the possibility of devising therapeutic strategies based on the druggability of these molecules. Recent efforts have focused on developing selective inhibitors for aurora and Polo kinases^{100–105}. CDK inhibitors have been also considered as relevant drug candidates for cancer therapy owing to their potential role in restoring control of the cell cycle^{106,107}. However, the first generation of CDK inhibitors, such as *flavopiridol* and *UCN-01* have not shown significant clinical advantages¹⁰⁷. Why have these CDK inhibitors displayed such modest activity in the clinic? Is it possible that these compounds have off-target effects that prevented them from

reaching therapeutic concentrations? Alternatively, and in view of the results obtained with gene-targeted mice, it is possible that these CDK inhibitors were not tested in the most appropriate tumour types. As discussed above, mouse tumour models have illustrated that ablation of cyclin D1 (and hence CDK4 activity) prevents breast cancer when driven by *ErbB2* and *Hras* oncogenes, but not those dependent on *Myc*- or *Wnt1*-driven pathways⁵⁹. Moreover, CDK4 inhibition may be effective to prevent *Myc*-induced skin tumours⁵⁵, suggesting that such dependence on CDK4 activity is dictated by the nature of the oncogenic mutations responsible for driving tumour growth as well as by the cellular context in which these mutations occur. These observations raise the possibility that different tumour types, depending on their pathogenic spectrum of mutations, may display different sensitivity to CDK inhibition. This concept should be taken into consideration when evaluating the new generation of CDK inhibitors currently undergoing clinical trials (TABLE 2).

Genetic interrogation of the cell cycle in mice indicates that CDK inhibitors are likely to produce certain toxicities by affecting the proliferation of those cells that require specific interphase CDKs to maintain tissue homeostasis. Some expected toxicities for CDK4 (diabetes), CDK2 (sterility) or CDK6 (mild anaemia) inhibitors may be acceptable for adult cancer patients. On the other hand, small molecules against CDK1 (REF. 19) and possibly against CDK7 (REF. 108) or CDK11 (REF. 109), may result in general toxicities not too different from those induced by available cytotoxics. It is therefore not surprising that promiscuous CDK inhibitors often display high toxicity in early clinical trials¹⁰⁷. In addition, these inhibitors may produce other side-effects by inhibiting less-studied kinases such as *CDK10* and *CDK11* (BOX 2). Loss of *CDK10* increases activity of the transcription factor ETS2 on the promoter of *RAF1*, increasing Erk–MAPK pathway activity and relieving *tamoxifen*-induced G1 arrest in breast tumours¹¹⁰. Likewise, partial inactivation of *CDK11* leads to tumour development in mice¹¹¹, suggesting that this protein is haploinsufficient for tumour suppression. Thus, non-specific inactivation of *CDK10* and *CDK11* by wide-spectrum CDK inhibitors may have undesirable toxic effects and/or limit therapeutic responses. Unfortunately, these two proteins are not frequently included in the panel of kinases used to test the specificity of CDK inhibitors.

Loss of CDK expression by germline or conditional ablation of their corresponding loci may not necessarily result in the same phenotypical consequences as pharmacological inhibition. The latter often leads to incomplete inhibition and retains expression of the target. The generation of novel strains carrying conditional knock-in mutations that allow expression of kinase-dead CDKs or non-activating cyclins⁵⁸ should provide better models than classical knockout approaches. These models should not only predict the consequences of target inhibition in an *in vivo* setting, but also greatly help to identify the non-mechanism-based as well as off-target effects common to most, if not all, kinase inhibitors.

Table 2 | **Representative specific CDK inhibitors in clinical trials (single-agent trials)**

Compound	Primary target(s)	Clinical trials	Sponsor
AG-024322	CDK1 CDK2 CDK4	Phase I, advanced cancer: NCT00147485 Discontinued (2007)	Pfizer
AT-7519	CDK1 CDK2 CDK4 CDK5 GSK3 β	Phase I/II, advanced or metastatic tumours: NCT00390117	Astex
P276-00	CDK1 CDK4 CDK9	Phase I/II, advanced refractory neoplasms: NCT00407498	Piramal
P1446A-05	CDK4	Phase I, advanced refractory malignancies: NCT00772876	Piramal
PD-0332991	CDK4 CDK6	Phase I, advanced cancer: NCT00141297	Pfizer
R547 (also known as Ro-4584820)	CDK1 CDK2 CDK4 CDK7	Phase I, advanced solid tumours: NCT00400296	Hoffmann-LaRoche
Roscovitine (also known as seliciclib and CYC202)	CDK2 CDK7 CDK8 CDK9	Phase II, non-small cell lung cancer, nasopharyngeal cancer, haematological tumours: NCT00372073	Cyclacel
SNS-032 (also known as BMS-387032)	CDK1 CDK2 CDK4 CDK7 CDK9 GSK3 β	Phase I, B-lymphoid malignancies: NCT00446342 Phase I, solid tumours: NCT00292864	Sunesis

CDK, cyclin-dependent kinase; GSK3 β , glycogen synthase kinase-3 β .

Future perspectives

Our increasing understanding of the molecular mechanisms responsible for cell cycle misregulation in cancer suggests that human tumours accumulate mutations that favour re-entry into the cycle of quiescent or slow-dividing progenitor cells, result in DNA replication checkpoint deregulation eventually leading to GIN, and deregulate the mitotic checkpoint contributing to CIN. However, the relative contributions of GIN and CIN to the different steps of neoplastic development, including invasion and metastasis, are mostly unknown. As originally proposed by Boveri for CIN¹¹², GIN might not be oncogenic *per se*, but may be a mediator that facilitates fixation of new oncogenic mutations. Indeed, CIN itself, and perhaps GIN, may inhibit tumour growth, probably owing to inefficient cell proliferation or induction of apoptosis^{98,113}. However, the presence of mutations in checkpoint regulators in human syndromes and the tumour-prone phenotype of mouse models defective for checkpoint proteins suggest a causal role for these mutations in tumour development.

The value of targeting GIN as a therapeutic approach in cancer is not clear. Even if we could find a way to restore genomic stability in tumour cells, it is quite possible that such instability is no longer required for tumour growth once the appropriate mutations are fixed, especially in advanced neoplasias. However, enhancing GIN may be detrimental for tumours, as cells may have

an upper threshold of tolerance for GIN that provides a window of opportunity for therapeutic approaches. For instance, BRCA1 or BRCA2 dysfunction resulting in deficient homologous recombination repair sensitizes tumour cells to the inhibition of poly(ADP-ribose) polymerase (PARP), a protein involved in base excision repair¹¹⁴. The inhibition of PARP in BRCA1- and BRCA2-defective cells is synthetic lethal, resulting in GIN, cell cycle arrest and subsequent apoptosis¹¹⁴. Indeed, PARP inhibitors are already being tested in clinical trials. Similar efforts may lead to increased CIN and reduced cell viability in tumour cells with a defective mitotic checkpoint. Further preclinical models are required to evaluate these complex interactions before these therapies reach the clinic.

In any case, considering the central role of CDKs in controlling cell cycle pathways, the therapeutic value of inhibiting their kinase activity deserves more detailed evaluation. Synthesis of more selective and potent inhibitors would be required before undergoing new rounds of clinical trials. Above all, it is essential to better understand why normal and tumour cells have specific requirements for individual interphase CDKs. Only then will oncologists be able to design clinical trials that will match the CDK specificity of the drug candidate with the appropriate tumour type, either on the basis of its cell type of origin or, more likely, on its pathogenic spectrum of resident mutations.

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DATABASES

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[NCT00141297](http://clinicaltrials.gov/ct2/show/study?term=NCT00141297&rank=1) | [NCT00147485](http://clinicaltrials.gov/ct2/show/study?term=NCT00147485&rank=1) | [NCT00292864](http://clinicaltrials.gov/ct2/show/study?term=NCT00292864&rank=1) | [NCT00372073](http://clinicaltrials.gov/ct2/show/study?term=NCT00372073&rank=1) | [NCT00390117](http://clinicaltrials.gov/ct2/show/study?term=NCT00390117&rank=1) | [NCT00400296](http://clinicaltrials.gov/ct2/show/study?term=NCT00400296&rank=1) | [NCT00407498](http://clinicaltrials.gov/ct2/show/study?term=NCT00407498&rank=1) | [NCT00446342](http://clinicaltrials.gov/ct2/show/study?term=NCT00446342&rank=1) | [NCT00772876](http://clinicaltrials.gov/ct2/show/study?term=NCT00772876&rank=1)

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[flavopiridol](http://www.cancer.gov/drugdictionary/flavopiridol) | [tamoxifen](http://www.cancer.gov/drugdictionary/tamoxifen) | [LCN-01](http://www.cancer.gov/drugdictionary/LCN-01)

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[ataxia-telangiectasia](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM&term=ataxia-telangiectasia) | [Li-Fraumeni syndrome](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM&term=Li-Fraumeni%20syndrome) | [mosaic variegated aneuploidy](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM&term=mosaic-variegated-aneuploidy) | [premature chromatid separation](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM&term=premature-chromatid-separation) | [Seckel syndrome](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM&term=Seckel-syndrome)

UniProtKB: <http://www.uniprot.org/>
[ATM](http://www.uniprot.org/ATM) | [ATR](http://www.uniprot.org/ATR) | [BRCA2](http://www.uniprot.org/BRCA2) | [BUB1](http://www.uniprot.org/BUB1) | [BUBR1](http://www.uniprot.org/BUBR1) | [CDC20](http://www.uniprot.org/CDC20) | [CDC6](http://www.uniprot.org/CDC6) | [CDC42A](http://www.uniprot.org/CDC42A) | [CDH1](http://www.uniprot.org/CDH1) | [CDK1](http://www.uniprot.org/CDK1) | [CDK10](http://www.uniprot.org/CDK10) | [CDK2](http://www.uniprot.org/CDK2) | [CDK4](http://www.uniprot.org/CDK4) | [CDK6](http://www.uniprot.org/CDK6) | [CENPF](http://www.uniprot.org/CENPF) | [CHK1](http://www.uniprot.org/CHK1) | [CHK2](http://www.uniprot.org/CHK2) | [CKS2](http://www.uniprot.org/CKS2) | [cyclin A1](http://www.uniprot.org/cyclinA1) | [cyclin A2](http://www.uniprot.org/cyclinA2) | [cyclin D1](http://www.uniprot.org/cyclinD1) | [cyclin D2](http://www.uniprot.org/cyclinD2) | [cyclin D3](http://www.uniprot.org/cyclinD3) | [cyclin E1](http://www.uniprot.org/cyclinE1) | [cyclin E2](http://www.uniprot.org/cyclinE2) | [geminin](http://www.uniprot.org/geminin) | [H2AX](http://www.uniprot.org/H2AX) | [INK4A](http://www.uniprot.org/INK4A) | [INK4B](http://www.uniprot.org/INK4B) | [INK4C](http://www.uniprot.org/INK4C) | [INK4D](http://www.uniprot.org/INK4D) | [MAD1](http://www.uniprot.org/MAD1) | [NEK2](http://www.uniprot.org/NEK2) | [p21](http://www.uniprot.org/p21) | [p27](http://www.uniprot.org/p27) | [p53](http://www.uniprot.org/p53) | [p57](http://www.uniprot.org/p57) | [PLK1](http://www.uniprot.org/PLK1) | [RAD21](http://www.uniprot.org/RAD21) | [RAD51](http://www.uniprot.org/RAD51) | [RASGRF1](http://www.uniprot.org/RASGRF1) | [RB](http://www.uniprot.org/RB) | [RBL1](http://www.uniprot.org/RBL1) | [RBL2](http://www.uniprot.org/RBL2) | [securin](http://www.uniprot.org/securin) | [SKP2](http://www.uniprot.org/SKP2) | [ITK](http://www.uniprot.org/ITK) | [SMAD3](http://www.uniprot.org/SMAD3) | [TPX2](http://www.uniprot.org/TPX2)

FURTHER INFORMATION

M. Malumbres' homepage: <http://www.cnio.es/ing/grupos/plantillas/presentacion.asp?grupo=50004262>

M. Barbacid's homepage: <http://www.cnio.es/ing/grupos/plantillas/presentacion.asp?grupo=50004258>

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Biographies

Marcos Malumbres obtained his Ph.D. in Biology from the Universidad de León, Spain, in 1993. He studied the relationship between Ras and cell cycle regulation in his postdoctoral work at the New York University Medical Center, New York, USA (1994–1998). He then joined the Spanish National Cancer Research Centre (CNIO) in Madrid where he has been Head of the Cell Division and Cancer Group since 2004. He currently focuses on the characterization of the *in vivo* function of mitotic regulators that have therapeutic implications in cancer.

Mariano Barbacid led a group that isolated the first human oncogene (*HRAS*) in 1982 and helped to establish its mechanism of activation. Since then, he has made important contributions to the role of oncogenes in cancer, for which he has received several international awards. After spending a number of years at the National Cancer Institute, Maryland, USA, he became Vice President of Oncology Drug Discovery at Bristol–Myers Squibb, Princeton, New Jersey in 1988. In 1998 he returned to Madrid, Spain, to create the CNIO, where he is the current Director and Head of the Experimental Oncology Group.

000 Cell cycle, CDKs and cancer: a changing paradigm

Marcos Malumbres and Mariano Barbacid

Misregulation of cyclin-dependent kinases (CDKs) can induce unscheduled proliferation and genomic and chromosomal instability. How has recent genetic evidence changed our understanding of the roles of CDKs in the cell cycle of normal and tumour cells?