PHYSIOLOGICAL RELEVANCE OF CELL CYCLE KINASES

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Malumbres M. Physiological Relevance of Cell Cycle Kinases. Physiol Rev 91: 973–1007, 2011; doi:10.1152/physrev.00025.2010.—The basic biology of the cell division cycle and its control by protein kinases was originally studied through genetic and biochemical studies in yeast and other model organisms. The major regulatory mechanisms identified in this pioneer work are conserved in mammals. However, recent studies in different cell types or genetic models are now providing a new perspective on the function of these major cell cycle regulators in different tissues. Here, we review the physiological relevance of mammalian cell cycle kinases such as cyclin-dependent kinases (Cdks), Aurora and Polo-like kinases, and mitotic checkpoint regulators (Bub1, BubR1, and Mps1) as well as other less-studied enzymes such as Cdc7, Nek proteins, or Mastl and their implications in development, tissue homeostasis, and human disease. Among these functions, the control of self-renewal or asymmetric cell division in stem/progenitor cells and the ability to regenerate injured tissues is a central issue in current research. In addition, many of these proteins play previously unexpected roles in metabolism, cardiovascular function, or neuron biology. The modulation of their enzymatic activity may therefore have multiple therapeutic benefits in human disease.

I. INTRODUCTION

Multicellular organisms require an adequate control of cell division and differentiation to coordinate multiple different cell types in functional tissues. The cell division cycle entails the tightly regulated transduction of mitogenic signals to a series of biochemical machineries that control the duplication of DNA and its proper segregation to daughter cells. The molecular basis of this regulation was first studied in landmark genetic screens in yeast (119, 247, 248). One of these cell division cycle (Cdc) genes, Cdc28 in Saccharomyces cerevisiae and Cdc2 in Schizosaccharomyces pombe, was identified as a protein kinase that regulated itself by phosphorylation (305). This kinase was subsequently found to be the active component of the mitosis-promoting factor, a proteinaceous factor that could drive the division of cells (103).

A number of different studies in yeast and other organisms have led to a model where cell cycle kinases act as major engines to promote progression throughout the different phases of the cell division cycle. These phases include two major periods of activity in which the genome is first duplicated (DNA synthesis or S phase) and the two newly replicated genomes are then distributed between the daughter cells (mitosis) (FIGURE 1). Additional gap periods, G1 (preceding S phase) and G2 (preceding mitosis), are required to coordinate DNA synthesis and segregation with mitogenic signals and to synthesize and assemble the required proteins and cellular structures. In addition, to ensure proper progression through the cell cycle phases, these molecular engines are under the control of internal checkpoints that monitor proper conditions to generate healthy daughter cells (120). Thus the cell division cycle can be defined as the process by which cells monitor proper conditions for cell division, activate the required biochemical machineries for DNA replication and chromosome segregation, and monitor these steps to generate two genomically stable daughter cells.

Regulation of the cell cycle is well conserved from yeast to humans, and most original Cdc genes have a mammalian counterpart. However, as it may be expected from the diversity of mammalian cell types, many of these individual yeast genes are represented as complex gene families in mammals. Among these regulators, cell cycle kinases are known to have a critical role not only as major regulators of cell cycle progression but also in tissue homeostasis and human disease (208). Because of their catalytic activity, these proteins are also considered as druggable targets, and new small-molecule inhibitors, mostly ATP competitors, are now in the pipeline of many pharmaceutical companies.

In this review, I focus not only on the relevance of mammalian cell cycle kinases during cell cycle progression but also
on the physiological regulation of different mammalian tissues. The complexity of this analysis can be predicted from the presence of multiple family members of many of these kinases in the mammalian genome (summarized in sect. II) and their differential expression and activity in the multiple cell compartments. Since cell proliferation is a major cellular process during development, the relevance of cell cycle kinases in the formation of tissues and in the regenerative potential from progenitor cells will be treated in section III. Their implication in different adult tissues and physiological functions will be further discussed in section IV. Finally, it is well established that cell cycle kinases are deregulated in proliferative diseases such as cancer. These alterations and the therapeutic implications of modulating cell cycle kinase activity in patients will be finally discussed in section V.

II. CONTROL OF THE CELL DIVISION CYCLE BY PROTEIN KINASES

A typical eukaryotic cell has to complete sequentially several important processes during the cell cycle. However, most adult cells are quiescent and do not express many cell cycle genes. Thus the first requirement for cell cycle entry relies on the activation of the transcriptional and translational machinery required for the specific expression of cell cycle genes (212). This machinery is thought to be active during early cell divisions in the embryo, but a repression mechanism becomes crucial for those cells that terminally differentiate during organogenesis. The repression program is likely to be cell type-specific, but it is generally represented by the retinoblastoma protein (pRb). pRb and the other pocket family members p107 and p130 are transcriptional regulators that repress a large list of genes including critical cell cycle regulators. This repression is commonly mediated through the recruitment of repressor complexes, such as the SWI/SNF complex, histone deacetylases, polycomb group proteins as well as methylases, and the binding and inactivation of transcription factors such as the E2F family (52, 206). Thus, in quiescent cells, the expression of genes required for cell cycle progression is frequently repressed in a pRb-dependent manner (212).

Numerous mitogenic signals are responsible for the inactivation of the pRb repression machinery through the activation of cyclin-dependent kinases (Cdks) (212, 223). These proteins are usually present in quiescent cells as inactive kinases due to the lack of their activators, the cyclins. Cyclins are indeed transcriptionally induced in response to mitogenic signals, leading to the activation of Cdks and phosphorylation of the pRb protein. This phosphorylation results in a massive transcription during G1 of genes required for the subsequent cell cycle phases (FIGURE 1). Some of these genes expressed during late G1 are necessary for activation of the DNA replication complexes during S phase. Other kinases participate in DNA replication itself (Cdc7) or in the duplication of centrosomes, a cellular struc-
ture that will be later required during mitosis. During G2, these centrosomes move towards different poles of the cell. In addition to some Cdk family members, other kinases are specifically dedicated to drive centrosome duplication and maturation and to increase their ability to nucleate microtubules during these stages, including Aurora and Polo-like kinases or the never in mitosis a (NIMA)-related kinases (Neks). The critical molecular activity required for entry into mitosis is Cdk1 (the ortholog to yeast Cdc28/Cdc2) kinase. During the G2/M transition, a large number of proteins are phosphorylated in a Cdk1-dependent manner resulting in cytoplasmic changes, Golgi disassembly, nuclear envelop breakdown, and condensation of chromosomes (211). Some other kinase activities, mainly from Aurora or Polo families, participate in mitotic entry by helping in the establishment of the proper chromosome structures and the mitotic spindle. In addition, a new kinase involved in the inactivation of phosphatases, known as Mastl, is thought to participate by favoring the kinase involved in the inactivation of phosphatases, helping in the establishment of the proper chromosome structures and the mitotic spindle. In addition, a new kinase involved in the inactivation of phosphatases, known as Mastl, is thought to participate by favoring the balance between Cdk1-dependent phosphorylation and dephosphorylation by general phosphatases such as protein phosphatase (PP) 2A. A particular group of kinases, represented by Bub1, BubR1 kinases and Mps1 (also known as TTK), are critical components of the spindle assembly checkpoint (SAC or mitotic checkpoint), a signaling pathway that monitors bipolar attachment of chromosomes to the mitotic spindle. This checkpoint ensures proper segregation of chromosomes to daughter cells. Finally, Aurora and Polo-like kinases are involved in cytokinesis, the process by which the cytoplasm divides to generate two independent daughter cells (FIGURE 1). Other kinases not directly involved in the regulation of cell cycle progression such as the enzymes involved in the DNA damage response (ATR/ATM or Chk1/Chk2) have been deeply reviewed (13, 51, 151, 272, 304) and will be no further discussed here.

A. Cyclin-Dependent Kinases

Not surprisingly, the number of Cdks has significantly increased during evolution. Up to six conserved Cdks exist in the budding yeast S. cerevisiae, although only one of them, Cdc28 (Cdk1), is necessary and sufficient to drive the cell cycle (28, 128). The nonessential Cdk Pho85 is also involved in the earlier phases of the cell cycle, whereas the other Cdks are thought to function mainly in transcription (136). Twenty different Cdks (Cdk1–20) exist in mammals (213). Only four Cdks, Cdk1, 2, 4, and 6 (and perhaps Cdk3 and Cdk5), are clearly associated with cell cycle control. Whereas a few additional Cdks, Cdk7–11, are involved in the control of transcription, some others do not have a clear function assigned yet (211, 213).

1. Mitotic Cdks

As indicated above, Cdk1 is the founding member of the Cdk family. In yeast, Cdk1 can bind and be activated by all interphase and mitotic cyclins (28). In mammals, Cdk1 has been typically associated with A-type (G2) and B-type (late G2 and mitosis) cyclins. However, recent studies have clearly demonstrated that Cdk1 can also bind interphase (D-type and E-type) cyclins in wild-type cells, and these interactions are enforced in cells deficient in the interphase Cdks (such as Cdk4 or Cdk2) that are commonly associated with these interphase cyclins (6, 215, 288). Cdk1 phosphorylates a wide spectrum of proteins including more than 70 targets validated in yeast (84) and mammals (211) (TABLE 1). This number is likely to be underestimated since massive chemical/proteomic studies have identified over 300 potential targets (129, 334). Through these posttranslational modifications, Cdk1 controls critical processes required for mitosis such as nuclear envelop breakdown, disassembly of the Golgi apparatus, chromosome cohesion and condensation, formation of the spindle, and attachment of chromosomes to the spindle. In addition to these mitotic processes, Cdk1 may also be crucial for regulating transcription as well as DNA replication and repair. These functions have been deeply studied in yeast given the abundant genetic tools in this organism (84). The in vivo requirements for Cdk1 in mammals have been much more difficult to analyze given the essentiality of this protein within a cell cycle and the lack of genetic models. Genetic ablation of Cdk1 in the mouse arrests embryos at the one-cell/two-cell stage (209, 288), and further analysis has been so far prevented by this early lethality.

Although no other Cdk seems to be able to complement the lack of Cdk1 in mammals, an additional family member, Cdk11, also plays critical roles during mitosis. Cdk11-null embryos die at the blastocyst stage due to the arrest of blastomeres in mitosis (190). Cdk11 may indeed act as a microtubule stabilizing factor that regulates centrosome maturation and spindle assembly (260, 363) and sister chromatid cohesion (133). Impaired translation of Cdk11 also leads to other multiple mitotic defects (350). Cdk11 is also known to play a role in transcription and RNA splicing (132, 201, 202).

2. Interphase Cdks

Whereas the yeast Cdk1 is able to regulate G1 and S phase through binding to interphase yeast cyclins, a variety of Cdks seem to have undertaken this role in mammals. Cdk4 and Cdk6 are relatively similar to yeast Cdc28 and Pho85 (209). These mammalian Cdks are activated by D-type cyclins (D1, D2, and D3), which are the major cell cycle sensors for mitogenic signals in the cell cycle. D-type cyclins are transcriptionally induced by virtually all mitogenic signals including major signaling routes such as the Ras/mitogen-activated protein kinase (MAPK) pathway. Upon induction of D-type cyclins, Cdk4 and Cdk6 become active and phosphorylate pocket proteins such as pRb, resulting in the inactivation of the repression machinery (212). Cdk4/6-cyclin D complexes are therefore major sen-
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Alias</th>
<th>Function</th>
<th>Regulatory Partners</th>
<th>Substrates (Vertebrates)</th>
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<td><strong>Cdk1 Cdc2a</strong></td>
<td></td>
<td>Mitosis (DNA replication and DNA damage, DNA repair)</td>
<td>Cyclins A, B (D and E)</td>
<td>Atpopaxin, adenomatous polyposis coli, Aki1 Amphiphysin 1, Anaphase Promoting Complex, Ase1, BARD1, Bcl-2, caldesmon, caspase-2, caspase-9, Cdc7, Cdc20, Cdc25A, Cdc25C, Cdh1, Cdk7, C/EBPβ, CKII, Ctip, dynamin, dystrophin, EF-1, Eg5, EGFR, ERK-3, FANC1, Fos, FoxM1B, FoxO1, Gfb-1, GFAP, GM130, GRASP65, histone H1, HHR6A, HMG-I(Y), IFAP300, KRC, lamin A, B, and C, lamin B receptor, Lats1, MAP1B, MAP4, Map205, Marcks, MCM2, MCM4, MKLP1, Myb, Nedd1, NBP60, neurofilament H, NF-I, Nir2, NO3B, nuclear pore complex, nucleolin, Nucks, numatrin, Orc1, p18, p47, p53, p54NRB, PAP, PHF8, plectin, PP1-δ, pRb, R2, Rab4, RapGAP, Raptor, RCC1, Rllalpha, RunX2, S6K1, Sam68, Securin, Separaose, Sirt1, Ski, survivin, mSt1, Tau, TMAP(CKAP2), vimentin, Vsp34, thymidine kinase, 53BP1</td>
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<td>G1/S transition, DNA damage, DNA repair</td>
<td>Cyclins E and A (also D), PCaf</td>
<td>ATRIP, BARD1, B-Myb, BRCA1, CBP/p300, Cdc6, Cdc7, Cdk7, Cdt1, C/EBPβ, DP1, HHR6A, HIRA, Ku70, Marcks, MCM2, MCM4, MyoD, Npat, nucleophosmin (B23), p107, p21cip1, p27kip1, p53, pRb, R2, RPA, Smad3, thymidine kinase</td>
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<td><strong>Cdk3</strong></td>
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<td>Cyclins E, A, and C, Cables</td>
<td>Cables 1, c-Jun</td>
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<tr>
<td><strong>Cdk4 PSK-J3</strong></td>
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<td>G1/S progression</td>
<td>D-type cyclins</td>
<td>Cdt1, Marcks, p107, p130, pRb, Smad3</td>
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<td><strong>Cdk5 TPKII</strong></td>
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<td>Neuron biology, senescence</td>
<td>p35, p39, cyclins D, E, and I</td>
<td>AATK1, Amphiphysin 1, Ape1, β2-Syntrophin, Cdk1, Disabled1, Doublecortin, eNOS, Munc18a, Nude, p53, PSD-95, Sds3, Stat3, Synapsin 1, tyrosine hydroxylase, Vsp34</td>
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<td><strong>Cdk6 PLSTIRE</strong></td>
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<td>G1/S progression</td>
<td>D-type cyclins</td>
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<td>Cyclin H</td>
<td>Cdk1, 2, 4, 5, 6, RNA pol II COOH-terminal domain</td>
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<td>eIF3, Pak1, RanBPM</td>
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<td>Centrosome and spindle function</td>
<td>Ajuba, Arpc1b, PAK1, Tpx2</td>
<td>Arpc1b, BRCA1, CENP-A, CPEB, Eg5, Histone H3, LATS2, Ndel1, N-Myc, p53, TACC</td>
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sors for cell cycle entry by inactivating the pRb-dependent repression machinery commonly present in adult cells. Why are there three different D-type cyclins and two different D-type cyclin-associated kinases? As we will see later, the different combinations of D-type cyclins and Cdks are likely to provide additional flexibility in different cell types or in response to different mitogenic signals.

Cdk2 and Cdk3 are highly similar to Cdk1 but do not share its mitotic functions. Both proteins associate to E- and A-type cyclins, and their activities may be involved in G₁ progression, entry into S phase, and DNA repair. Cdk3 is expressed at low levels in mammals, and its function remains to be clearly established (211). Cdk2 is also able to phosphorylate pRb, and it may perform similar functions to Cdk4 in the control of cell cycle entry (212). In addition, it may participate in DNA damage and DNA repair, although many of these functions are also shared by Cdk1 (49, 69, 74, 142, 241).

Cdk5, the mammalian Cdk with the highest similarity to yeast Pho85, is a very particular protein as it is activated by noncyclin proteins, p35 and p39, that are almost uniquely expressed in brain. Cdk5 can also bind D-type and E-type cyclins, although the activity of these complexes is unclear. Cdk5 has multiple roles in neuron biology including neuronal migration and survival, dendrite extension, and synapsis (76, 156). Cdk5 also display additional func-

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### Table 1—Continued

<table>
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<td>Mitosis</td>
<td>Activated by Nek9</td>
<td>Nek6, Nek7</td>
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<tr>
<td>Mastl</td>
<td>Greatwall, FLJ14813, Thc2</td>
<td>Inhibition of PP2A, mitosis</td>
<td>Activated by Cyclin B/Cdk1</td>
<td>No substrates identified</td>
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tions in cell adhesion (327) and cellular senescence in non-neuronal cells (219).

3. Cdk-activating kinases and transcriptional Cdks

Mammalian Cdk7 is a component of the Cdk-activating kinase (CAK) which phosphorylates and activates several cell cycle Cdks. In addition, CAK is part of the general transcription factor TFIIH involved in promoter clearance and progression of transcription (94). Cdk20 (Ccrk) is highly related to Cdk7 both in sequence and function, and it may also phosphorylate and activate other Cdks such as Cdk2 (7, 244). It may regulate primary ciliation in some model organisms (175).

Cdk7–11 also function as transcriptional regulators, in some cases with direct links to cell cycle control (211). Thus Cdk7 is a component of the general transcription factor TFIIH, whereas Cdk8, Cdk9, and Cdk11 regulate RNA polymerase II, among other functions (211). The Cdk8–highly related kinase Cdk19 is likely to have similar functions as a component of the mediator complex (213, 292). No clear function has been assigned to Cdk10. Similarly, Cdk12–Cdk18 play diverse functions in the control of diverse cellular processes, including the regulation of the retinoblastoma pathway, although the relevance of these kinases is mostly unknown (213).

B. Aurora Kinases

Early work in Drosophila led to the identification of aurora mutants, which carry a loss-of-function mutation in a serine/threonine kinase essential for centrosome separation and the formation of bipolar spindles (108). A single Aurora protein exists in budding (increase-inploidy 1; Ipl1) or fission (Ark1) yeast, whereas two family members, Aurora A and Aurora B, are present in worms, flies, and frogs. Three different Aurora family members, known as Aurora A, B, and C, exist in mammals (245). These kinases contain a conserved catalytic domain and NH2-terminal domains that vary in sequence and in length. Aurora B and C are close paralogues that probably arose from a relatively recent common ancestor (34).

Aurora kinases participate in multiple processes during the mammalian cell cycle (45). Aurora A, the ortholog to the original Drosophila kinase, localizes on duplicated centrosomes from the end of S phase to the beginning of the following G1 phase, when this kinase is degraded by the proteasome in an APC/C-Cdh1-dependent manner. Aurora A participates in several processes required for building a bipolar spindle including centrosome separation and microtubule dynamics (17, 106) (Figure 1). Aurora B, on the other hand, belongs to the chromosome passenger complex (CPC) that localizes to the kinetochores from prophase to metaphase and to the central spindle and midbody in cyto-kinesis (45, 279). The CPC is responsible for the recruitment to the kinetochore and centromere of a growing number of proteins including inner centromeric proteins, regulators of the microtubule-kinetochore, or proteins involved in the SAC (168). Some of these molecules, such as the depolymerizing factor MCAK, are Aurora B substrates, suggesting a critical role for the CPC in the destabilization of aberrant microtubule-to-kinetochore attachments and the SAC-dependent delay in mitotic progression until these defects are corrected (279). Recent data suggest that substrate phosphorylation depends on the distance of the substrate from Aurora B at the inner centromere, thus indicating that recruitment of the CPC to the kinetochore prevents the stabilization of improper attachments and activates the SAC to delay the metaphase to anaphase transition (195). During cytokinesis, Aurora B is localized to the midbody remnant where its local inactivation is crucial for completion of abscission (115, 311). Aurora B also participates in mitotic phosphorylation of Ser-28 and, probably, Ser-28 in histone H3, and it may regulate cell differentiation through these epigenetic modifications (281).

Less is known about Aurora C, which is expressed at high levels in the testis (45). Aurora C can bind members of the CPC (191), and its ectopic expression can rescue Aurora B loss of function in cultured cells (291, 307, 359), suggesting a potential function as a regulator of microtubule-kinetochore interactions, at least in some cell types. Although Aurora C is known to have a specific role in spermatogenesis (77, 172, 321), its relevance in the regulation of mitosis is not well understood.

C. Polo-Like Kinases

Polo was originally identified in Drosophila as a mutant with mitotic and meiotic defects in the microtubule spindle (317). This mutation was later mapped to a serine-threonine protein kinase specifically concentrated in dividing cells (204). Polo-like kinases (Plks) are found in all eukaryotes and are characterized by a highly related kinase domain, and one or two COOH-terminal polo box domains (PBDs), involved in subcellular localization and partner interaction (83, 338). These include the Plk1 subfamily, containing Drosophila polo and mammalian Plk1, the SAK subfamily, containing Drosophila SAK and mammalian Plk4, both of which are widely distributed across the eukaryotes, and the Plk2 subfamily, containing vertebrate Plk2 and Plk3, and also including homologs from echinoderms (15, 18, 218). The mammalian genome contains a fifth member of the Plk family, Plk5, initially described as a pseudogene (39) and recently linked to DNA damage (9) and neuron biology (72). Although mouse cells express a full-length Plk5 similar in size to Plk2 or Plk3, human cells express a shorter Plk5 form in which the kinase domain is disrupted due to a stop mutation in exon 6 which is followed by an in-frame ATG codon immediately down-
The founding member of the family, Plk1, localizes to the cytoplasm and centrosomes in interphase and concentrates to the kinetochores and the cytokinesis bridge during cell division. This protein has major functions in centrosome maturation, mitotic entry, and cytokinesis (15, 18, 261, 320). The other members of the family are less studied. Plk4 (Sak) is a critical regulator required for centriole duplication both in Drosophila and mammals (25, 116). Plk2 (also known as Snk) localizes to the centrosome and may also participate in centrosome biology and S-phase checkpoints (227). Plk3 (Fnk or Prk) activity peaks in G1 and localizes to the nucleolus in interphase. This protein may function in S-phase entry (374), and it is activated in response to repressive stress and genotoxic insults leading to apoptosis in a p53-dependent manner (345, 356, 357).

The three Plk subfamilies have distinct functions and operate in multiple cell types. Their expression is regulated differentially in cells and tissues and in response to several cellular processes and stimuli (352). Whereas Plk1 and Plk4 are found only in dividing cells, Plk2, Plk3, and Plk5 are also expressed in neurons and other, nondividing, differentiated cells (72, 299, 339). Plk2 and perhaps Plk3 seem to have a crucial function in modulating synaptic plasticity in neuronal dendritic spines through the phosphorylation of the spinde-associated protein SPAR (10, 298; and see below). Plk5, on the other hand, is involved in neuron differentiation and growth of neurites (72).

D. Checkpoint Kinases: Bub1, BubR1, and Mps1

The SAC is a highly conserved cellular mechanism that ensures chromosome segregation fidelity in all eukaryotes by delaying anaphase onset in response to kinetochores that are unattached to microtubules. There are more than 14 proteins that may be involved in SAC signaling, and at least four of them are protein kinases: Bub1, BubR1, Mps1, and Aurora B, although Aurora B may be strictly dispensable for SAC function (240). Plk1 displays a relevant activity in recovery to DNA damage checkpoint (340), and it may also be involved in some aspects of the SAC, although its relevance is not clear (63). As long as there are unattached kinetochores or improper attachments unable to produce enough tension, the SAC is active and some SAC components such as BubR1 sequester and inhibit Cdc20, the activating subunit of the APC/C. Upon complete attachment and biorientation of the chromosomes, the SAC is satisfied and APC/C-Cdc20 becomes active, resulting in the ubiquitination of securin and cyclin B that are subsequently degraded in a proteasome-dependent manner (216, 240).

1. Bub1 and BubR1

The budding uninhibited by benzimidazole (Bub) proteins were originally characterized in S. cerevisiae screens for mutants that were unable to recover from a transient treatment with a microtubule inhibitors (131). Bub1 is a protein kinase that first localizes at the forming kinetochore during prophase, and it is required for the recruitment of other proteins to the kinetochore. Bub1 plays several roles in regulating kinetochore assembly, spindle function, as well as sister chromatid separation (220). Bub1 monitors microtubule attachment to the kinetochores by phosphorylating Mad1 or Bub3, thus modulating the availability of Mad2 to inhibit APC/C-Cdc20. Cdc20 is also a direct target of Bub1 resulting in APC/C inhibition. Bub1 also regulates centromeric cohesion by direct phosphorylation of threonine 120 (serine 121 in fission yeast) of the conserved C-tail of human H2A. This phosphorylation is crucial for centromeric localization of shugoshin, since most defects observed after inhibition of Bub1 are phenocopied in H2A mutants in which this residue has been changed to alanine, and these defects are suppressed by tethering shugoshin proteins at centromeres (166). The shugoshin-PP2A complex protects centromeric cohesin during mitotic prophase by avoiding excessive phosphorylation and premature release of cohesins. Bub1 is therefore crucial for establishing a centromeric mark for shugoshin localization and the proper control of sister chromatid separation (166).

In general, Bub1 seems to regulate the switch from lateral to end-on attachment, while BubR1 is required for stabilization of kinetochore-microtubule attachments (220). Aurora B has an additive effect on the misalignment phenotype of Bub1-depleted cells but a suppressing effect in the BubR1 phenotype, suggesting that the defective attachments in Bub1-deficient cells are detected by active Aurora B, which acts to destabilize them, thereby creating the possibility for new correct attachments to be established.

2. Mps1 (Ttk)

Mps1, from the monopolar spindle phenotype in the yeast (351) (also known as Ttk in mammals), is a dual-specific protein kinase that may play specific roles in centrosome duplication and cytokinesis (95). However, the kinetochore functions of Mps1 have been studied the most extensively. Mps1 is required for the SAC due to its requirements for kinetochore recruitment of several checkpoint proteins, including Mad1 and Mad2 (2, 96, 126, 198, 207, 221, 287, 314, 324). In the absence of Mps1, the SAC is not functional, and cells rapidly exit from mitosis even in the presence of microtubule poisons. Mps1 may also play additional roles in the regulation of p53 (139) and Abl (246).
E. Additional Cell Cycle Kinases: Cdc7, Neks, and Mastl

Progression throughout the cell cycle involves multiple molecular and cellular processes, and a number of additional kinases are involved in the control of different aspects of the mammalian cell cycle. For instance, more than 40 human kinases result in specific mitotic phenotypes when repressed by RNA interferences as reported by the MitoCheck Consortium (243) (http://www.mitosys.org/cgi-bin/mtc?query=kinase&query_type=genes). These include Aak1, Acr1C, Cdc2L5, Cdk15, Cit, Dak, Gak, Gsk5, Kdrn, Lck, Limk1, Lmtk3, Map2k5, Map3k2, Mastl, Mst4, Nek3, Nek10, Nek9, Nttrk3, Pak2, Pask, Pdk1, Pink1, Pkn3, Prkab1, Prkacb, Prkce, Prkcv, Prkg1, Prky, Ptk7, Ret, Ripk4, Rps6ka2, Ryk, Sgk223, Stk16, Stk39, Tesk1, Tssk3, Ulk4, Vrk1, Vrk2, Wee1, and Wnk4. Other kinases with certain relevance in the cell cycle include the replication kinase Cdc7 (see below) or other proteins such as Tousled-like kinases (Tlk1 and Tlk2), Lats kinases, and the Cdk inactivating kinases Weel/Myt1 or Haspin (Gsg2) that have been reviewed elsewhere (127, 167, 208, 275, 333). In some cases, the link between these kinases and the cell cycle may be indirect or we do not have a clear picture of how their function modulates cell cycle progression. However, a few of these kinases, such as Cdc7, Neks, or Mastl, have a clear direct role in the cell cycle, and they are also known to have physiological relevance in some specific tissues in vivo.

Chromosome replication is a highly complex process that occurs once in every cell cycle in most cells. A conserved kinase called Cdc7 cooperates with Cdns in establishing replication forks during the initiation of chromosome replication. Cdc7 is required for efficient S phase progression by phosphorylating the Mcm2–7 helicase and relieving an inhibitory activity residing within the Mcm4 NH2-terminal domain (35, 68, 98, 301, 302). Cdc7 may also have additional roles in the DNA damage response, mitosis, or meiosis (162, 170, 225, 231, 330), although these putative functions have not been fully studied in mammals.

Neks represent a family of serine/threonine kinases named after the NIMA gene originally cloned in Aspergillus nidulans. Eleven mammalian family members (Nek1-Nek11) maintain significant homology with the A. nidulans NIMA protein, although the COOH-terminal noncatalytic region is highly divergent, suggesting that each family member may have distinct functions (249). Only four of these proteins, Nek2, Nek6, Nek7, and Nek9, have been shown to play relevant roles in mitotic progression. Nek2, the closest relative to NIMA, localizes to the centrosome and plays a role in establishing the bipolar spindle through initiating the separation of centrosomes and contributing to microtubule organization at the G2/M transition by phosphorylating several centrosomal substrates (TABLE 1) (249). Nek2 may also play additional roles in chromosome condensation and the SAC, through its interaction with Hec1 and Mad1, Nek9, Nek6, and Nek7 function in a kinase cascade that participates in the formation and/or maintenance of the mitotic spindle (20). Nek9 phosphorylates both Nek6 and Nek7, relieving an inhibitory signal and thus activating these two kinases (274). In general, Nek kinases seem to play a critical role in microtubule organization, not only during mitosis but also in cilia (253, 268). In fact, Nek1 and Nek8 have been implicated in cilia function, since these genes are mutated in mouse models of polycystic kidney disease (197, 336).

Recently, a new kinase known as Mastl in mammals and Greatwall in Xenopus and Drosophila has been shown to be critical for maintaining the mitotic state. Greatwall was originally identified as a kinase required for chromosome condensation (365). Recent studies indicate that Mastl/Greatwall inhibits PP2A in complex with the B55 regulatory subunit, a complex that can remove the phospho-residues established by Cdk1 during mitosis. However, the activity of Mastl inhibits PP2A, ensuring that Cdk1 substrates remain phosphorylated during mitosis (38, 48, 341). Interestingly, the human MASTL gene is mutated in a novel form of autosomal dominant inherited thrombocytopenia (102).

III. CELL CYCLE KINASES IN DEVELOPMENT AND STEM CELL FUNCTION

A. Development

Given the strong requirements for cell division during development, it is not surprising that many cell cycle kinases are critical for the formation of adult mammals. The generation of mouse models with loss-of-function mutations in specific genes has produced in the last years a significant amount of data on the requirements for these proteins in different stages of development or in different tissues (TABLE 2).

1. G1/S kinases: reentering the cell cycle in the embryo

Perhaps the most unexpected result in the late 1990s and early 2000s was the relative dispensability of the major G1/S kinases: Cdk4, Cdk2, and Cdk6 during mouse development (23, 50, 215, 251, 270, 332). Even the combined ablation of two or the three of these genes only results in specific phenotypes in the fetal liver or in the heart, indicating that these proteins are not required for most cell divisions during development (209, 288). Given the high number of mammalian Cdns, it is still possible that the activity of these proteins is compensated by Cdk1 (288) or other non-well-characterized Cdns, such as the Cdk14 subfamily, that also bind D-type cyclins (213). However, the most
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Genotype</th>
<th>Phenotype</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cdk1</td>
<td>Cdk1\textsuperscript{mut} [gene trap]</td>
<td>Early embryonic lethality (2-cell stage)</td>
<td>288</td>
</tr>
<tr>
<td></td>
<td>Cdk1\textsuperscript{Cdk2KI} [Cdk2 cDNA into Cdk1 locus]</td>
<td>Early embryonic lethality</td>
<td>293</td>
</tr>
<tr>
<td>Cdk2</td>
<td>Cdk2\textsuperscript{(-/-)}</td>
<td>Viable. Male and female sterility.</td>
<td>23, 251</td>
</tr>
<tr>
<td></td>
<td>E\textsubscript{\textmu}Myc; Cdk2\textsuperscript{(-/-)}</td>
<td>Cdk2 is required for Myc-induced lymphomas</td>
<td>43</td>
</tr>
<tr>
<td>Cdk3</td>
<td>Cdk3\textsuperscript{Stop/Stop} point mutation</td>
<td>Spontaneous strain. No phenotype</td>
<td>362</td>
</tr>
<tr>
<td>Cdk4</td>
<td>Cdk4\textsuperscript{(-/-)}</td>
<td>Viable. Diabetes and hypopituitarism due to defective proliferation of endocrine cells. Delayed entry into S phase in MEFs</td>
<td>270, 332</td>
</tr>
<tr>
<td></td>
<td>MMTV-Ras; Cdk4\textsuperscript{(-/-)}</td>
<td>Cdk4 is required for H-Ras-induced breast tumors</td>
<td>367</td>
</tr>
<tr>
<td></td>
<td>K-Ras V12; Cdk4\textsuperscript{(-/-)}</td>
<td>Cdk4 is required for K-Ras-induced lung tumors</td>
<td>266</td>
</tr>
<tr>
<td>Cdk6</td>
<td>Cdk6\textsuperscript{(-/-)}</td>
<td>Viable. Mild anemia</td>
<td>215</td>
</tr>
<tr>
<td>Cdk11</td>
<td>Cdk11\textsuperscript{(-/-)}</td>
<td>Embryonic lethality (blastocyte stage). Mitotic arrest and apoptosis. Haploinsufficient for skin tumor suppression</td>
<td>57, 190</td>
</tr>
<tr>
<td>Cdk2/4/6</td>
<td>Cdk2\textsuperscript{(-/-)}; Cdk4\textsuperscript{(-/-)}; Cdk6\textsuperscript{(-/-)}</td>
<td>Embryonic lethal (E13.5-E15.5) due to hematopoietic defects</td>
<td>288</td>
</tr>
<tr>
<td>AurkA</td>
<td>Aurka\textsuperscript{(-/-)}</td>
<td>Embryonic lethality (blastocyte stage). Mitotic arrest and monopolar spindle formation</td>
<td>53</td>
</tr>
<tr>
<td>AurkB</td>
<td>Aurkb\textsuperscript{(-/-)}</td>
<td>Lethality in postimplantation embryos due to rapid mitotic exit and apoptotic cell death</td>
<td>Fernandez-Miranda et al., unpublished observations</td>
</tr>
<tr>
<td>AurkC</td>
<td>Aurkc\textsuperscript{(-/-)}</td>
<td>Viable. Minor defects in sperm functionality (note that multiple AurkC loci may exist in the mouse genome)</td>
<td>172</td>
</tr>
<tr>
<td>Plk1</td>
<td>Plk1\textsuperscript{mut} [gene trap]</td>
<td>Early embryonic lethality (morula stage) due to interphase arrest. Haploinsufficient for tumor formation</td>
<td>203</td>
</tr>
<tr>
<td></td>
<td>Plk1\textsuperscript{(-/-)} and Plk1\textsuperscript{mut} [gene trap]</td>
<td>Early embryonic lethality (morula stage) due to mitotic arrest and the formation of monopolar spindles. Haploinsufficient for tumor formation</td>
<td>Wachowicz et al., unpublished observations</td>
</tr>
<tr>
<td>Plk2</td>
<td>Plk2\textsuperscript{(-/-)}</td>
<td>Viable. Retarded growth and skeletal development. Delayed entry into S phase in MEFs</td>
<td>205</td>
</tr>
<tr>
<td>Plk3</td>
<td>Plk3\textsuperscript{(-/-)}</td>
<td>Viable. Increased weight and increased susceptibility to tumor formation</td>
<td>361</td>
</tr>
</tbody>
</table>
likely explanation for the lack of embryonic phenotype in these models is that early embryonic cell cycles do not require interphase Cdk activity at all. Mammalian stem cells display a short G1 phase and a rapid alternation between S and M phases (250, 349), suggesting that early embryonic cells do not require the G1/S machinery. In fact, pRb is not expressed until midgestation, thus leading to a model where early cell divisions do not have an active repression machinery and G1 kinases are therefore not required for entering into the cell cycle (209). These kinases would only be required in those tissues where differentiation and the acquisition of a regulated cell cycle exit and entry is required. No wonder, the hematopoietic system is one of such systems in which proliferation and differentiation are tightly regulated to produce all types of hematopoietic cells required for embryo survival (215, 288).

Although the information is not as detailed for other interphase proteins, the observations made with Cdns suggest that other interphase kinases should also be dispensable for early cell divisions. Plk2, a member of the Polo-like kinase family with no role in mitosis, is also to be dispensable for embryonic development, although genetic ablation of Plk2 results in retarded growth and skeletal development late in gestation (205). Similarly, Plk3 is dispensable for embryonic development, although Plk3-deficient mice display increased susceptibility to tumor development (361). On the other hand, the replication protein Cdc7 is essential for embryonic development, and Cdc7-null embryos die between E3.5 and E6.5 due to cessation of DNA synthesis within S phase (171).

2. Mitotic kinases

Most kinases involved in centrosome biology or mitosis seem to be required for embryonic development. Genetic

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Genotype</th>
<th>Phenotype</th>
<th>Reference Nos.</th>
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<tbody>
<tr>
<td>Plk4</td>
<td>Plk4(−/−)</td>
<td>Mitotic arrest and postgastrulation (E7.5) defects during embryonic development. Haploinsufficient for suppression of liver tumors</td>
<td>141, 176</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bub1</td>
<td>Bub1(−/−)</td>
<td>Embryonic lethality (E3.5) due to proliferative arrest and apoptosis</td>
<td>257</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bub1hypomorph</td>
<td>Increased aneuploidy and susceptibility to spontaneous tumors (liver and lung tumors, sarcoma and lymphoma)</td>
<td>154</td>
</tr>
<tr>
<td>BubR1</td>
<td>BubR1b(−/−)</td>
<td>Embryonic lethality (E8.5). Abnormal megakaryopoiesis in heterozygous mutants</td>
<td>346</td>
</tr>
<tr>
<td></td>
<td>BubR1bhypomorph</td>
<td>Prone to aneuploidy, premature ageing, infertility, increased susceptibility to induced tumors</td>
<td>16, 70, 271</td>
</tr>
<tr>
<td>Cdc7</td>
<td>Cdc7(−/−)</td>
<td>Embryonic lethality between E3.5 and E6.5 due to S-phase defects. Partially rescued in a p53 background</td>
<td>171</td>
</tr>
<tr>
<td>Nek1</td>
<td>Spontaneous loss-of-function mutations</td>
<td>Polycystic kidney disease</td>
<td>336</td>
</tr>
<tr>
<td>Nek7</td>
<td>Nek7(−/−)</td>
<td>Late embryonic development of perinatal lethality. Increased ploidy in MEFs.</td>
<td>282</td>
</tr>
<tr>
<td>Nek8</td>
<td>Spontaneous loss-of-function mutation</td>
<td>Polycystic kidney disease</td>
<td>197</td>
</tr>
</tbody>
</table>
ablation of Cdk1, the master mitotic kinase, results in a
dramatic arrest at the two-cell stage, indicating the strong
requirement for de novo synthesis of this kinase during
embryonic development (FIGURE 2). Cdk11 deficiency also
results in early embryonic lethality due to mitotic arrest and
apoptosis of the blastocyst (190). Aurora A and Plk1 are
also required for early divisions as their absence results in
arrest at the morula or early blastocyst stage [about embry-
onic day (E)3.5; Refs. 53, 203]. In both cases, the absence of
these kinases results in mitotic arrest (53; P. Wachowicz, G.
de Carcer, and M. Malumbres, unpublished information),
suggesting the essentiality of these two proteins in mitotic
progression. Genetic disruption of Plk4 results in embry-
onic lethality by E7.5, leading to an accumulation of mitotic
and apoptotic cells during gastrulation (141).

In mammals, the CPC seems to be also required for
early embryonic development before implantation. Dege-
eration of embryos is apparent by E2.5-E3.5 in the absence
of the CPC components Survivin (337), Incenp (56), or
Borealin (358). In these models, several mitotic defects are
observed including abnormal mitotic spindles and variable
nuclear sizes as a consequence of defective cytokinesis.
However, Aurora B-null embryos develop normally during
the early cell divisions and die only after implantation, sug-
gest ing the presence of normal cell cycles in the early em-

3. Checkpoint kinases and genomic instability

The SAC is not essential in budding yeast or in Dro-
sophila probably because chromosome attachment to mi-
crotubules is very rapid and efficient (37, 107). However,
all SAC genes studied to date are essential in mice, including
Bub1 and BubR1 (FIGURE 2). Bub1-null mice die shortly
after day E3.5, and this protein is required at all stages
analyzed during embryonic development (257, 325). On
the other hand, embryos homozygous null for Bub1b (the

![FIGURE 2](http://physrev.physiology.org/)

Requirements for cell cycle kinases during embryonic development in the mouse. The embry-
onic stage mostly affected by the genetic ablation of single kinases is indicated. Some kinases dispensable
dfor embryonic development display specific defects in adult tissues or in tumor development (see text and
TABLE 2 for details).
gene encoding BubR1) die by E8.5 in utero (346). Although the molecular and cellular phenotype has not been characterized in detail in these embryos, this lethality is accompanied by proliferation defects and massive apoptosis. Genetic disruption of the murine Ttk (Mps1) locus has not been reported yet. However, Mps1 is required for development in zebrafish, and a hypomorphic mutation in the Mps1 gene in this organism results in frequent developmental aneuploidy as a consequence of impaired checkpoint activity in germ cells (262, 263).

B. Stem Cell Function and Asymmetric Cell Division

From the previous section, it may be concluded that some cell cycle kinases, such as Cdk1, Plk1, or Aurora A, are essential for cell division in every cell type analyzed (including early embryonic cells), whereas checkpoint kinases such as Bub1, BubR1, and Mps1 may be dispensable within a few cell cycles but their inhibition results in chromosomal instability and apoptotic cell death. Although not tested directly yet, these essential roles are supposed to apply similarly to stem cells. G1/S kinases, however, may have special relevance in stem cell biology, since the presence or not of G1 or the length of this phase may be a major regulatory factor in adult stem cell function. Adult stem cells maintain tissue function and integrity throughout an organism’s lifespan by replacing differentiated cells as they are lost because of attrition or damage. To perform this function, stem cells are endowed with a unique combination of pluri-lineage developmental potential and the capacity to enter the cell cycle when required. In addition, these cells usually have the ability to perform two different cell divisions: symmetric, to produce two identical stem cells; or asymmetric, in which cell division results in a stem cell and a differentiated (or committed to differentiation) cell.

1. Regulation of G1 phase in stem/progenitor cells

Embryonic stem cells (ESCs) exhibit a short G1 phase that significantly lengthens as cells differentiate during development. In the embryonic epiblast, the mean generation time has been estimated to be as low as ~5 h and correlates with the high Cdk activity observed in cultured ESCs (349). These early embryos are likely to lack a functional pRb pathway and cell-cycle-dependent transcription as observed in ESCs (310, 349). As embryonic cells differentiate, the cell cycle is likely to become dependent on specific transcriptional waves required for expressing S phase and mitotic regulators, resulting in a prominent G1 phase or in a quiescent (G0) state.

Less information is available on how adult stem cells regulate the entry into the cell cycle upon proper stimulation. These cells are typically quiescent or slow-proliferating and are therefore quite different from ESCs under a cell cycle perspective. In fact, recent genetic studies in the mouse have revealed the importance of stem cell quiescence in the maintenance of stem cell function during adulthood (250). Some cell cycle kinases are likely to participate in this regulation. In fact, the phenotype of hematopoietic stem cells (HSCs) deficient in the Cdk inhibitor p21Cip1 was one of the first indications suggesting the relevance of cell proliferation in stem cell exhaustion. In p21-null mice, the percentage of HSCs in G0 is reduced in agreement with increased proliferative potential of these cells. As a consequence, the bone marrow in these animals is rapidly exhausted after serial transplantation or myelotoxic agents (62). Similar results in the increased reduction of p21-null neurosphere-initiating cells with ageing (174) suggest a general mechanism by which stem cells need to maintain under tight control the number of cell divisions throughout life. The fact that p21Cip1 is a major Cdk inhibitor suggests that Cdns are major players in controlling the balance between stem cell quiescence and proliferation. In fact, similar results have been obtained after genetic disruption of other unrelated Cdk inhibitor, p16Ink4a, when depleted in young HSCs (153). Interestingly, ablation of p16Ink4a in old HSCs displays the opposite effect and improves the regenerative potential of these cells, as well as neuronal or pancreatic stem/progenitor cells (153, 179, 232). Similarly, genetic ablation of p18Ink4c results in increased number and function of HSCs (364, 368). These results are difficult to conclide in a single model at this moment, although they may indicate different functions of the target kinases (Cdk1/2 for p21Cip1 and Cdk4/6 for Ink4 inhibitors) in ageing or the response to stress (250). For instance, Cdk2-null cells from the subventricular zone (SVZ) display decreased self-renewal capacity and enhanced differentiation (149). Cdk4 may compensate for the lack of Cdk2 in young animals, but not in old mice (149), in agreement with the age-related increase of Ink4 inhibitors that may limit Cdk4 function with age. Cdk4 is also a central kinase in triggering cell division in skin stem cells. In quiescent hair follicle stem cells, Cdk4 is repressed by the nuclear factor of activated T cells c1 (NFATc1). As stem cells become activated during hair growth, NFATc1 is downregulated, relieving Cdk4 repression and activating proliferation of these cells (130).

All together, these studies suggest that the G1 phase is a sensitive period during which cell-fate decisions are made with the help of crucial signals from the microenvironmental niche of the adult stem cell population (250). G1 Cdns and their regulatory subunits may play a critical role in maintaining stem cell function through life, and the alteration in this control is likely to lead to multiple pathological abnormalities (FIGURE 3).

2. Asymmetric versus symmetric cell division in stem/progenitor cells

Neural stem cells are a good model to study the relevance of symmetric versus asymmetric cell division in
mammals. As development proceeds, an increasing proportion of neural stem cells start to switch from divisions that generate additional stem cells (symmetric cell divisions) to divisions that generate committed progenitors or postmitotic cells (asymmetric cell divisions) (294, 372). This switch leads to the generation of neurons and intermediate progenitors that leave the ventricular zone and form a second germinal region, the SVZ, which is thought to be important for increasing cortical surface.

It has been recently proposed that the length of the G$_1$ phase plays a central role in the switch from symmetric to asymmetric cell division in these neural precursors. Overexpression of Cdk4/cyclin D1 in neural progenitors shortens G$_1$ by 30% inhibiting neurogenesis (asymmetric cell divisions that generate one stem/progenitor cells plus a differentiated neuron) and promotes the expansion of basal progenitors through symmetric cell divisions (185). On the other hand, partial inhibition of Cdks induces premature neurogenesis (42). Although formal demonstration of this hypothesis remains to be provided, these results suggest that the observed lengthening of G$_1$ is not only a consequence of differentiation but may also play causal roles in inducing neurogenesis and differentiation (283).

In addition to the role of interphase Cdks in regulating G$_1$, the balance between symmetric and asymmetric cell divisions may be also modulated by other cell cycle kinases involved in centrosome or spindle function. In vivo, the decision between symmetric or asymmetric cell divisions is likely to be modulated by external signals from the stem cell niche that determine the orientation of the cell division. In general, daughter stem cells will remain in the niche, whereas the differentiated cell will migrate out of the niche. This has been well documented at least for two mammalian systems: the skin and the nervous tissue. In the skin, the basal epidermal cells can divide asymmetrically by orientating the spindle perpendicularly to the basement membrane, a process in which...
integrins and cadherins are essential for the apical localization of atypical protein kinase C (aPKC), the Par3-Lgn-Inscuteable (Ins) complex, and NuMA-dynactin to align the spindle (186).

However, as indicated above, most information on asymmetric cell division comes from the study of neuroblasts in flies (90, 111, 113, 144, 265). The initial evidence of a direct link between cell cycle kinases and asymmetric cell division comes from the functional analysis of Cdk1 in Drosophila. An original screen identified defects in asymmetric cell division in neuroblasts caused by a specific mutation (E51Q) in Cdk1 (326). The premature attenuation of Cdk1 activity in this mutant results in mislocalization of both apical (Ins, partner of Ins/Lgn and Bazooka/Par3) and basal (Prospero, Brat, and Miranda) asymmetric markers (FIGURE 4). The second mitotic kinase implicated in asymmetric divisions was Aurora A (21, 344). A hypomorphic allele of Aurora A impairs asymmetric localization of the basal asymmetric marker Numb and increases the number of symmetric cell divisions. These defects are thought to be caused by altered dynein-dependent spindle orientation and delocalization of specific cortical markers such as aPKC or Numb recruited through the Aurora A-dependent phosphorylation of Dlg(Pins), Par-3, and Par-6 (158, 169, 187, 353). Aurora B may also play a role in asymmetric cell division as its CPC partner INCENP is required for the asymmetric segregation of Prospero during asymmetric neuroblast division in Drosophila (58). Finally, the Drosophila Polo has been shown to directly phosphorylate partner of Numb (Pon), a Drosophila-specific adaptor protein that regulates the asymmetric distribution of Numb (343). In Polo mutants, the asymmetric distribution of Pon, Numb, and aPKC are disrupted resulting in the expansion of neuroblasts at the expense of neurons. Despite the relevance of these studies in flies, the relevance of cell cycle kinases in neural stem cells in mammals remains mostly unexplored (36).

FIGURE 4  A model for the control of neuroblast proliferation and asymmetric cell division by cell cycle kinases. In the embryonic neuroepithelium, apical neuroblasts divide symmetrically to produce two progenitors (left) or asymmetrically to generate one progenitor and one differentiated neuron (right). The decision may be modulated by Cdk4 through the control of the length of the G1 phase of the cell cycle, by several microcephaly proteins (such as Aspm and Cdk5Rap2) that regulate centrosome function, and by mitotic kinases by regulating the localization of apical (green area) or basal (orange) markers. The involvement of mitotic kinases has not been demonstrated in mammals and comes from studies in flies. Aspm, Cdk5Rap2, and the kinase Cdk19 are mutated in human microcephaly syndromes.
IV. AN INTEGRATIVE VIEW OF CELL CYCLE KINASES IN TISSUE FUNCTION AND DISEASE

From the original studies in yeast or invertebrates, it is obvious that cell cycle kinases are critical regulators required for cell cycle progression in cultured cells. Their relevance in mammalian development is also expected, and their putative roles in stem cells further support the relevance of these kinases for proliferation and homeostasis of adult tissues. However, despite the critical importance of proper cell cycle regulation in establishing the correct morphology of tissues during development, little is known about how the cell cycle is regulated in a tissue-specific manner. In the next sections, we will focus on several tissues whose function is known to be modulated by specific cell cycle kinases. The wide variety of cell types within an adult mammal requires important variations in the cell cycle, from differentiated cells that maintain certain potential to proliferate (such as endocrine cells or certain hematopoietic cells at various stages of maturation), to specific tissues that required giant cells generated by endoreduplication or lack of cytokinesis (e.g., megakaryocytes) or by hypertrophic growth (cardiac cells). In some cases, cell cycle kinases play critical roles independent of the cell cycle function as it is clearly the case in specific postmitotic neurons. Some clinical data also suggest that specific human diseases may be caused by alterations in the function of specific cell cycle kinases, and these include specific pathologies in the indicated tissues as well as the generation of aneuploidy in the germ cells due to the alteration of checkpoint kinases during meiosis. The role of cell cycle regulators in other tissues or cell types not considered here, e.g., muscle (44, 342) or vascular cell function and lesions (8, 101), has been previously reviewed.

A. Endocrine Tissues, Adipogenesis, and the Control of Metabolism

One of the first links between cell cycle regulation and the homeostasis of different tissues in vivo was provided by heterozygous mice with a targeted mutation in the gene encoding pRb. Whereas complete ablation of pRb results in embryonic lethality due to placental, hematopoietic, and neuronal defects, pRb(+/−) mice develop frequent pituitary tumors as well as other endocrine hyperplasias and neoplasias (150). As expected, many of these phenotypes were reproduced in Cdk4(R24C) knockin mice expressing a hyperactive form of Cdk4 that results in increased pRb inactivation (270, 309). These mice develop pituitary tumors as well as other endocrine pathologies such as insulinosomas or Leydig cell tumors. Conversely, Cdk4 deficiency results in specific defects in endocrine tissues, mostly endocrine pancreas, the pituitary, and Leydig cells in the testis, whereas the rest of the tissues are mostly unaffected in the absence of Cdk4 (270, 332) (FIGURE 5). In addition, endocrine tissues are frequently altered in most mouse models with specific mutations in G1/S regulators such as cyclins or Cdk inhibitors such as p27Kip1 or p18Ink4c (269). The reason for the special sensitivity of endocrine tissues to the alteration of cell cycle regulators is unclear, although it may be somehow related to relevance of G1 regulation in maintaining the proliferative potential of endocrine progenitors.

![Figure 5](http://physrev.physiology.org/)}
In agreement with this hypothesis, most spontaneous endocrine tumors display aberrations in cyclins, Cdk5, or Cdk inhibitors, in addition to the inactivation of pocket proteins (269). In addition, a germ line nonsense mutation on the human CDKN1B gene encoding p27kip1 has been associated with the development of a multiple endocrine neoplasia-like syndrome (255).

Cdk4-deficient mice die of diabetes due to defective proliferation of postnatal pancreatic beta cells, whereas the expression of the hyperactive Cdk4 R24C results in insulinoma (270). Cyclin D1 or D2 specifically activate Cdk4 to promote proliferation of pancreatic beta cells and to facilitate the activation of the proper progenitors in the ductal epithelium (182, 188). This function is probably mediated by the pRb-E2F pathway as E2f1/E2f2-double knockout mice display similar insulin-deficient diabetes accompanied by reduction in the number and size of pancreatic islets (147). Cdk6 is not expressed in these cells in the mouse (thus explaining the strong phenotype in Cdk4-deficient mice; Ref. 222), whereas it seems to be a major component of the G1/S machinery in human pancreatic β-cells (92). Reexpression of Cdk6 in mouse pancreatic β-cells using transgenic mice mostly rescues the pancreatic defects in Cdk4-null mice (J. Martín and S. Ortega, personal communication), thus suggesting the compensatory role between these two kinases.

Is the relevance of the Cdk4-E2F pathway in pancreatic β-cells exclusively due to their effect on proliferation? A recent study suggests that this is not the case. Cdk4, cyclin D1, and E2F1 proteins are highly expressed in nonproliferating pancreatic β-cells and may control insulin secretion through transcriptional regulation of Kir6.2 (12). Kir6.2 is a direct E2F1 target gene and plays a major role in the regulation of insulin secretion by controlling membrane polarization. Upon high blood glucose levels, Cdk4 is activated resulting in pRb phosphorylation, activation of E2F1, transcriptional induction of Kir6.2, and secretion of insulin (12). Since glucose is required for proliferation of most cell types, this mechanism suggests a “obligatory” link between cell cycle molecules and control of metabolism (27).

The general implications of Cdk4 in the control of glucose homeostasis and oxidative glycolysis have been recently reviewed (27). However, these connections are likely to affect other cell cycle kinases. When the cell enters the cell cycle, enormous changes take place in catabolic and anabolic processes to facilitate duplication of the genome and biosynthesis of cellular structures and organelles. It is therefore expected that cell cycle kinases may have direct or indirect roles in controlling enzymes required for biosynthesis. As an example, the yeast Cdk1 phosphorylates Pho2, a transcription factor involved in the expression of purine and histidine biosynthesis genes, as well as Tgl4 and Smp2, two proteins involved in fatty acid synthesis (181, 194, 289). In mammals, the Cdk4/pRb/E2F pathway is also known to regulate adipogenesis through complementary mechanisms. Cdk4 directly promotes adipogenesis by phosphorylating pRb and activating E2F function (1). E2F transcription factors regulate adipogenesis through modulation of the expression of the nuclear receptor PPARγ, which is a well-established master regulator of adipogenesis (88). PPARγ and pRb form a repressor complex with the histone deacetylase HDAC3, and this complex is dissociated after phosphorylation of pRb by Cdk5, resulting in adipocyte differentiation (87). In addition, the direct phosphorylation of PPARγ by Cdk5 leads to dysregulation of a large number of genes whose expression is altered in obesity, and it may be therefore involved in the pathogenesis of insulin resistance (64).

B. Hematopoiesis

Hematopoiesis is a durable process throughout the lifespan of an animal. As discussed above, the proliferative potential of hematopoietic stem and progenitor cells must be tightly regulated to maintain the capacity of producing the proper cell populations upon a variety of conditions. The control of proliferation by G1/S regulators during hematopoiesis has been deeply reviewed (312). In general, maturation and terminal differentiation are accompanied by an increase in Cdk inhibitors and an overall falloff in Cdk activities. In many cases, this is required for pRb-mediated terminal differentiation through its effects on tissue-specific transcription factors such as GATA-1, the master transcription factor of erythropoiesis (161, 273), or in mitochondrial biogenesis (284).

Among the Cdks, Cdk6 seems to play specific roles in the hematopoietic system (FIGURE 5). Cdk6-deficient mice display specific alterations in erythropoiesis and T-cell function, whereas all other tissues display normal morphology and function (135, 215). Conversely, knockin mice expressing a hyperactive Cdk6 mutant (Cdk6 R31C) insensitive to Ink4 inhibitors develop T-cell hyperplasia and lymphoma (E. Rodriguez, V. Quereda, and M. Malumbres, unpublished information). Cdk6 has been proposed to play specific roles in preventing hematopoietic differentiation that are not shared by Cdk4 (114). In fact, Cdk6 may directly inhibit transcriptional activation by Runx1, a transcription factor required for the opening of chromatin of important hematopoietic regulator genes, thus inhibiting myeloid lineage-specific gene expression and terminal differentiation. Interestingly, this function does not require Cdk6 kinase activity and is therefore independent on pRb function (100).

Not only G1 kinases are able to modulate the hematopoietic system. The inhibition of mitotic kinases, which is lethal in
How cells regulate polyploidization or endomitisos is not clear at present, although several evidences suggest that specific mitotic kinases may be inhibited to allow normal S phases without concomitant mitosis or without cytokinesis. As described above, most of these evidences come from in vitro assays, and further in vivo information from mouse models will be required. The only addition to this scenario from human disease comes from a syndrome of thrombocytopenia characterized by abnormal platelet numbers in affected patients. This syndrome has been recently linked to a mutation in Mastl (102), a kinase involved in the maintenance of mitotic Cdk-dependent phosphosites through inhibition of the Cdk-counteracting PP2A (48, 341). In fact, in vivo inactivation of Mastl in zebrafish results in defective polyploidization (138) and, in fact, BubR1(+/−) mice display an increase in the number of splenic megakaryocytes and megakaryocytic progenitors in bone marrow cells. This enhanced megakaryopoesis in BubR1(+/−) mice is not completely functional due to a defect in the formation of proplatelet-producing megakaryocytes (346).

In postnatal hearts, the predominant form of growth is not cell division but an increase in cell size (hypertrophy). This change in the regulation of the cell cycle is not well understood but is preceded by a round of acytokinetic mitosis in which cardiac myocytes generate two nuclei after chromosome segregation without cytokinesis. Thus it is generally assumed that adult cardiomyocytes have very limited potential for self-renewal, and this is a major factor that prevents proper repair of the mammalian heart after injury. Most G1 cyclins and Cdks are not expressed in adult cardiomyocytes and, conversely, several Cdk inhibitors are present in these cells (4). Entry into the cell cycle after injury or re-expression of cell cycle proteins frequently leads to DNA replication and increase in ploidy. However, cytokinesis is not observed under these circumstances. The molecular basis for this defect is not well understood, and the activity of cytokinesis kinases (FIGURE 1) has not been properly tested in these cells. Only Plk1 has been shown to be downregulated in the adult heart (105). The decrease in activation of the Rho GTPase pathway, a target of Plk1 and Aurora B during cytokinesis, may also be responsible for the defects in the formation of the actomyosin ring in these adult cells (4).

These data may have relevance in the clinic as hypertrophic growth is a common pathology in humans. This hypertrophic growth is commonly accompanied by the upregulation of G1 cyclins and Cdks and the cyclin D-Cdk4/2-pRB pathway is likely to regulate cardiac myocyte size and hypertrophic growth (11, 193, 373). Interestingly, this process is also accompanied by the activation of the other class of Cdks, the transcriptional Cdks such as Cdk9. Cdk9 phosphorylated the COOH-terminal domain of RNA polymerase II, and its activity is derepressed in a number of models of cardiac hypertrophy conferring a predisposition to heart failure (285, 286).

The therapeutic application of all this information is still in its infancy. On one hand, the overexpression of cyclin D2, but not D1 or D3, in a mouse model of myocardial infarction promotes cell cycle entry and cardiac function (121, 254), suggesting that cell cycle-based strategies aimed to
increase Cdk4/2 activity can be exploited to drive myocardial repair following injury. On the other hand, the overactivation of some of these kinases, such as Cdk9 (and perhaps Cdk4/2), may lead to hypertrophic growth and, in fact, inhibition of Cdk9 may have potential therapeutic value (347). In addition, the CAK-related kinase Cdk20 promotes cardiac growth and has been found to be specifically downregulated in heart failure after myocardial infarction (267).

D. The Nervous System and Neuron Function

The role of cell cycle kinases in the proliferation of neural progenitors has been described above (see sect. III). Both the G1/S machinery (Cdk2, -4, and -6) and the mitotic kinases possibly involved in neural asymmetric cell division (Cdk1, Aurora A, and Plk1) may modulate the development of the nervous system and the production of new neurons from progenitor cells. In terminally differentiated neurons, reentry into the cell cycle frequently triggers neuronal death instead of proliferation, which may be related to some acquired and neurodegenerative disorders. In neurodegenerative diseases such as Alzheimer’s disease, Parkinson’s disease, amyotrophic lateral sclerosis, and Niemann-Pick disease type C, some populations of neurons complete DNA synthesis, but the cell cycle is arrested at the G2/M transition (99). Whether inhibition of these cell cycle kinases may protect adult neurons or progenitor cells from entering and/or progressing through the cell cycle thus preventing neurodegeneration is an attractive hypothesis that deserves further research in the upcoming years.

Microcephaly is a human syndrome characterized by reduced brain size and mental retardation that may be caused by mutations in at least five known genes (MCPH1–5) (323). Interestingly, all proteins encoded by these genes are centrosomal, although the significance of this finding is still unknown. MCPH3 is also known as Cdk5Rap2 (or Cep215), a protein that binds Cdk5 at the centrosome (277). In addition, at least two of the MCPH proteins, Aspm (MCPH5) and Cdk5Rap2, are regulated by Plk1 (79, 112, 118). Recently, Cdk19 has also been identified as the target of an inversion in chromosome 6 that leads to microcephaly and mental retardation in human patients (239). These data suggest putative functions of Cdk5 or Plk1, in addition to Cdk19, in mammalian neuroblasts (FIGURE 4), and the implications of these kinases in microcephaly deserve further research efforts.

In addition to the implication of cell cycle kinases as regulators of cell proliferation in the developing or regenerating nervous system, recent observations have proposed new roles for these regulators in neuron function that are totally uncoupled from cell division. The pionneer work on Cdk5 led to the identification of this protein as a kinase critical for correct migration during cortical development (55, 76). Cdk5 is mostly expressed and active in postmitotic neurons that express p35 and p39, the noncyclin activators of Cdk5. Cdk5 is therefore mostly inactive in proliferating cells despite the fact that it can bind D-type cyclins and phosphorylate the pRb protein upon specific conditions. A number of studies have uncovered critical functions for Cdk5 in neurite growth, neuronal survival, and synaptic plasticity both in developmental and adult neurogenesis, and this protein has been proposed as a therapeutic target in neurodegenerative diseases (156, 328). Cdk5 also suppresses the neuronal cell cycle by sequestering E2F1 into Cdk5-p35-E2F1 complexes that are transcriptionally inactive and therefore disrupting the active E2F1-DP1 complexes (370). This anti-cell cycle activity is most likely a neuroprotective function of Cdk5. Although no other Cdk5s have been shown to share these functions, it is worth noting that some less-studied Cdk5s, such as Cdk16–18, may also interact with the p35 activator subunit (213), and Cdk20 is subjected to specific epigenetic regulation in the brain (89).

Both Plk2 and Plk3, the lesser known Polo-like kinases, participate in neuron structure and synaptic plasticity, and the conserved Polo-box-domain (PBD) is involved in this neuronal function (163). Plk2 and Plk3 mRNAs increase in response to neuronal activity, and both proteins bind to Cib, a calcium- and integrin-binding protein. Plk2 specifically controls the protein levels of Spine-associated Rap guanosine triphosphatase activating protein (SPAR), and this mechanism is thought to be essential for homeostatic plasticity, i.e., the normalization of synaptic activity to within an optimal range in the face of chronic excitation or depression (252). As in the case of Plk1 substrates, SPAR needs to be primed by other kinases. Whereas the major priming kinase for Plk1 is Cdk1, Cdk5 performs a similar role in neurons by phosphorylating SPAR and priming it for Plk2-dependent phosphorylation (298). Thus Plk2 is a critical kinase for plasticity of hippocampal neurons during epileptiform activity and chronically elevated activity (298, 300). Plk2, as well as Plk1 and Plk3, also phosphorylate the neuronal α-synuclein, a phosphoprotein accumulated in several neurological diseases such as Parkinson’s and dementia with Lewy bodies (148, 228). Plks specifically colocalize with phosphorylated α-syn in primary neurons as well as in α-syn transgenic mice, especially cortical brain areas involved in synaptic plasticity. Furthermore, Plk2 is significantly increased in brains of Alzheimer’s disease and Lewy body disease patients (228). The newest member of the Plk family, Plk5, is mostly expressed in the adult murine and human brain and may modulate neurite formation in established cell lines and primary neurons (72). As suggested for Plk2 and Plk3, this function is also likely to be dependent on the PBD as human Plk5, which does not contain the kinase domain, also modulates neurite formation. Thus it is tempting to speculate that Plk5 has evolved as a kinase-deficient Plk-
family member whose activity mostly depends on protein-protein interactions mediated by the PBD (72).

Whereas Aurora B expression seems to be restricted to proliferating cells, Aurora A is expressed in the hippocampus and is enriched at the postsynaptic compartment. The stimulation of glutamate receptors results in Aurora A activation and phosphorylation of CPEB-1, a protein involved in translation by direct binding to specific elements in the mRNAs. This activation leads to increased polyadenylation and translation of CaMKII, a mechanism with potential implications in hippocampal long-term potentiation (140). More recently, the activity of Aurora A has also been linked to microtubule dynamics of postmitotic neurons. In dorsal root ganglia neurons, aPKC phosphorylates Aurora A resulting in increased autophosphorylation of this kinase and binding to its activator Tpx2. Aurora A-Tpx2 complexes localize to the spindle where Aurora A subsequently phosphorylates the peptidase Ndel1, a protein that associates with dynein and promotes neurite outgrowth (235, 236).

Because many mitotic kinases regulate centrosome and microtubule dynamics (required for the formation of the mitotic spindle), it is possible that these proteins may also regulate microtubule dynamics in neurons, given the relevance of this process in axon and dendrite function. It is not surprising therefore that the single polo kinase present in yeast has evolved to form four additional paralogs, three of which display specific neuronal functions. In addition, several Nek kinases are specifically expressed in the nervous system (14), and Nek3 seems to be especially relevant to regulate microtubule acetylation in neurons (59).

E. Germ Cells and the Generation of Aneuploidy

From a physiological point of view, the production of aneuploidy in specific somatic cell types does not have major consequences (unless these alterations are perhaps combined with other mutations that may lead for instance to cancer; see sect. V). However, chromosome segregation fidelity is particularly important in meiosis and during development when the embryo undergoes rapid cellular divisions, since these defects may lead to aneuploid individuals. Aneuploidy is the most common genetic disorder affecting human reproduction, and ~25% of human zygotes generated are thought to be aneuploid (122). These embryos are usually lost as spontaneous abortions, and only trisomies of chromosomes 13, 18, or 21 and aneuploidies of the sex chromosomes are compatible with life. It is well known that these abnormalities correlate with maternal age, and several cell cycle kinases and specifically checkpoint kinases are thought to play critical roles in preventing these defects.

All mitotic kinases discussed here (e.g., Polo, Aurora, or checkpoint kinases) play also major roles during meiosis. The case of Aurora C is particularly interesting as the expression of this protein is mostly restricted to germ cells and early developing embryos (22, 329, 360; G. Fernández-Miranda, M. Trakala, I. Pérez, M. Malumbres, unpublished data). Genetic ablation of murine Aurora C results in partial sterility due to minor defects in sperm morphology (172), although the interpretation of this phenotype may be complicated by the presence of multiple Aurora C copies in the mouse genome (134). Microinjection of a kinase-deficient Aurora C mutant into mouse oocytes causes multiple defects, including chromosome misalignment, abnormal kinetochore-microtubule attachment, premature chromosome segregation, and cytokinesis failure in meiosis I. In addition, histone H3 phosphorylation and kinetochore localization of Bub1 and BubR1 are inhibited, suggesting a significant impairment in CPC function (360). Interestingly, a homozygous mutation (c.144delC) in the human Aurora C gene leads to meiosis I arrest or the production of large-headed multiflagellar polyploid spermatozoa in patients showing sterility (77, 78). These data suggest that Aurora C is a critical regulator of genomic instability during meiosis despite its limited activity in mitotic cells.

The alteration of mitotic checkpoint kinases also results in significant abnormalities during meiosis. BubR1 haploinsufficiency causes progressive aneuploidy, along with a variety of progeroid features, and both male and female mutant mice have defects in meiotic chromosome segregation and are infertile (16). On the other hand, perturbation of the kinetochore localization or activity of Bub1 leads to acceleration of meiosis I and increase in aneuploidy in female germ cells associated with the premature separation of sister chromatids (189, 229). Mutation in a single copy of Bub1 is sufficient to produce this phenotype, and these defects are stronger in female mice with advanced age (189). Both Bub1 and Mad2 phenotypes in aneuploidy are stronger in oocytes than in sperm. Although the molecular reason behind this observation is not clear, these data clearly establish the relevance of reduced SAC function in oocytes as a function of age and the consequences in pregnancy loss in humans (220). As described above, a hypomorphic mutation in Mps1 also results in reduced checkpoint activity and aneuploid progeny in zebrafish (263), although not in vivo models for this protein have been described in mammals.

V. CELL CYCLE KINASES AND CANCER

A plethora of studies on the cyclin-Cdk-pRb pathway in human tumors have led to the proposal that G1/S regulation is altered in almost every human tumor. More than 80–90% of tumors from different origin display altered Cdk4/6/2 kinase activity (212). As described in the previous sec-
tions, the hyperactivation of these G1 kinases may lead to increased proliferation of differentiated cells that otherwise divide very rarely (e.g., endocrine cells), or the increased entry into the cell cycle of cells that were terminally differentiated. In addition, we now understand that the capacity for self-renewal of progenitor/stem cells presents a risk to the organism since diverse pathologies, including cancer, may be induced if this property is not properly controlled.

The involvement of centrosomal and mitotic kinases has been controversial, since the initial studies failed to find mutation in these genes in clinical samples. The essential role for most of these kinases in the cell cycle indicates that complete loss-of-function mutations (homozygous null mutations or whole gene deletions) are not likely to occur in cancer cells as these alterations would be deleterious for tumor growth. On the other hand, tumor-associated overexpression is common for several of these genes (73, 258), although the causal effect of this alteration in tumor growth is always difficult to evaluate. More recently, additional specific point mutations or polymorphisms have been described in several cell cycle kinases (TABLE 3), and only the most relevant or new data and its significance in cancer and therapy will be discussed below.

A. Oncogenes or Tumor Suppressors?

1. Cdns

Extensive data on the alteration of G1 Cdns in cancer, mostly Cdk4 and Cdk6, are already available (209, 211, 212). The causal involvement of other Cdns in cancer development is not well documented. Cdk1 is overexpressed in multiple tumors (FIGURE 6), and it is part of the genetic signature associated with chromosomal instability in tumors (47, 258). Some transcriptional Cdns have been proposed as cancer targets due to its relevance in transcription (Cdk9) or in the activation of the other Cdns (Cdk7), but they are not commonly altered in human tumors. Cdk8 has been recently proposed as a critical oncogene in colorectal cancer by regulating β-catenin activity (93, 237). Cdk8 represses E2F1 activity protecting β-catenin/TCF-dependent transcription from inhibition by E2F1, thus explaining why colorectal tumors, which depend on β-catenin transcription for their abnormal proliferation, keep pRb intact (237). Among the less studied Cdns, Cdk20 has been shown to function as an oncogene in glioblastoma (244), ovarian carcinoma (355), and colorectal cancer (7). Cdk20, which is highly similar to Cdk7, supports proliferation in these tissues by regulating Cdk2, cyclin E, and pRb function, probably through its Cdk-activating kinase activity.

2. Aurora

Overexpression of Aurora A and B has been observed in several tumor types and has been linked with a poor prognosis of cancer patients (104, 258). Overexpression or amplification of Aurora A has been identified in breast, lung, head and neck, and colon cancers. Aurora B is similarly overexpressed in lung tumors, glioblastoma, and oral squamous cell carcinoma (FIGURE 6). However, perhaps the most interesting information about Aurora A and tumor development was generated in a genetic screen for skin tumor susceptibility genes in the mouse. Aurora A was identified as a low-penetrance tumor susceptibility gene in mouse and human tumors (85), and specific polymorphisms for Aurora A have been later described in multiple tumor types such as hepatocarcinoma and prostate, pancreas, colorectal, lung, gastric, breast, and esophageal cancer (5, 54, 61, 86, 160, 173, 199, 224, 230, 316). A polymorphism in Aurora B may also modulate tumor outcome in breast tumor patients (322). Finally, a mutation in the gene encoding Aurora C has also been described in lung tumors (71).

Although the molecular basis for the effect of these polymorphisms in cell cycle progression is mostly unknown, this information suggests that minor changes in kinase function in critical mitotic regulators may have relevant implications in tumor development. Typical molecular pathology techniques currently used in the clinic are not likely to identify these alterations, and the way is now open for genetic association studies.

3. Polo-like kinases

Plk1 is also overexpressed in several tumor types (FIGURE 6), and it is currently considered as a relevant oncogene and cancer target (73, 313) (see below). However, an interesting observation about the oncogenic or tumor-suppressor role of Polo-like kinases comes from genetic and epigenetic studies in the other Plk family members. Loss of heterozygosity (LOH) occurs at the Plk4 locus in 50% of human hepatocellular carcinomas (HCC) and is present even in preneoplastic cirrhotic liver nodules, suggesting that Plk4 may function as a tumor suppressor gene. This alteration is associated with reduced Plk4 expression in HCC and Plk4(+/-) cells display a high incidence of multinucleation, supernumerary centrosomes, and a near-tetraploid karyotype, probably as a consequence of defective RhoA signalling and cytokinesis failure (176, 278). Yet, the human Plk4 gene is overexpressed in multiple neoplasias (FIGURE 6), and Plk4 overexpression is likely to result in similar defects in genomic stability. Plk4 may also be silenced by promoter hypermethylation in HCC (256). Similarly, Plk2, Plk3, and Plk5 may also be considered as tumor suppressors as they are silenced by epigenetic means in glioblastoma as well as hematopoietic and liver tumors (72, 256, 319). In fact, overexpression of Plk2, -3, -4, and -5 results in cell cycle inhibition and apoptosis in the case of Plk5, suggesting the possible therapeutic value of reexpressing these proteins in tumors (72, 256). In addition, Plk3-deficient mice develop increased susceptibility to spontaneous tumors, further suggesting a tumor suppressor role for this protein (361). It is interesting to note that only a few point mutations have

MARCOS MALUMBRES

Physiol Rev • VOL 91 • JULY 2011 • www.prv.org
### Table 3  Alteration of cell cycle kinases in human disease

<table>
<thead>
<tr>
<th>Human Gene</th>
<th>Human Chromosome</th>
<th>Genetic or Epigenetic Alteration</th>
<th>Disease</th>
<th>Reference Nos.*</th>
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<tr>
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<td></td>
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<tr>
<td>CDK2</td>
<td>12. q13.2</td>
<td>P45L</td>
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<td>Cosmic</td>
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<td>CDK6</td>
<td>7q21.2</td>
<td>P199L and fs</td>
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<td>Cosmic</td>
</tr>
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<td>translocation</td>
<td>Hematopoietic disorders</td>
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<td>CDK8</td>
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<td>R424C; D189N</td>
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<td>Cosmic</td>
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<td><strong>Polo-like kinases</strong></td>
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<td>PLK1</td>
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<td>Carcinoma cell lines</td>
<td>Simizu et al., 2000</td>
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<td>2q13</td>
<td>Several point mutations and hypermethylation</td>
<td>Several tumor types(colorectal, lung thyroid, T cell leukemia)</td>
<td>303 Reviewed in Ref. 258, Cosmic</td>
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<td>Mosaic variegated aneuploidy and premature chromatid separation syndromes</td>
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<td>fs mutations in A9 and A7 repeats</td>
<td>Gastric, pancreas, ovary and colorectal tumors</td>
<td>3, 46 Cosmic</td>
</tr>
<tr>
<td><strong>Replication kinases</strong></td>
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<td>G119E and fs</td>
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<td>9q33.3</td>
<td>I99S</td>
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<td>Cosmic</td>
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<tr>
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<td>1q31.3</td>
<td>I275M and ns</td>
<td>Spontaneous mutations in lung and ovary tumors</td>
<td>Cosmic</td>
</tr>
</tbody>
</table>
been found in the Plk1 locus in tumor cells. Interestingly, these mutations result in increased Plk1 protein instability (306). Plk1(+/-) mice also display increased susceptibility to tumor development (203; P. Wachowicz, G. de Cárcer, and M. Malumbres, unpublished information), thus raising the question whether Plk1 may also function as a tumor suppressor.

4. Mitotic checkpoint kinases

All the three mitotic checkpoint kinases, Bub1, BubR1, and Mps1, are overexpressed in multiple tumor types (FIGURE 6). Yet, all these three kinases display loss-of-function mutations in human tumors. Bub1 mutations were originally found in a pioneer analysis of mitotic checkpoint genes in tumors with microsatellite instability (40). Multiple genetic and epigenetic alterations have been described in Bub1 and BubR1 in a variety of human cancers (258). Mutations in BubR1 are associated with the cancer-susceptible disorders mosaic variegated aneuploidy (MVA) and the premature chromatid separation syndrome (117, 226). These individuals are predisposed to develop childhood cancer including rhabdomyosarcoma, Wilms tumor, or leukemia (117). Cell lines derived from MVA patients with biallelic mutations have an impaired mitotic checkpoint, chromosome alignment defects, and low overall BubR1 abundance, resulting in premature sister-chromatid separation and aneuploidy (29, 315). Both Bub1 and

Table 3—Continued

<table>
<thead>
<tr>
<th>Human Gene</th>
<th>Human Chromosome</th>
<th>Genetic or Epigenetic Alteration</th>
<th>Disease</th>
<th>Reference Nos.*</th>
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<td>L621F, R282Q and fs,</td>
<td>Spontaneous mutations in ovary, skin and stomach tumors</td>
<td>Cosmic</td>
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<tr>
<td>NEK9</td>
<td>14q24.3</td>
<td>P870S and fs</td>
<td>Spontaneous mutations in lung and ovary tumors</td>
<td>Cosmic</td>
</tr>
</tbody>
</table>

Phosphatase-inhibiting kinases

| MASTL | 10q12.1 | CHECK | Thrombocytopenia; Spontaneous mutations in stomach and lung tumors | Cosmic |

* Cosmic database: http://www.sanger.ac.uk/genetics/CGP/cosmic.

FIGURE 6 Expression of cell cycle kinases in human cancer. Red cells indicate overexpression, whereas downregulation is indicated by blue cells for each tumor type. The figures within the cells indicate the number of studies where these changes are statistically significant \((P < 0.0001)\) versus the total number of studies including the corresponding probe. Cell color is determined by the best gene rank percentile for the analysis within the cell. Data are from Oncomine (www.oncomine.org). No significant differences were found for Plk5 (LOC126520) or Nek8 under these criteria. Other kinases not indicated in the figure were not analyzed.
BubR1 hypomorphic mice display increased susceptibility to develop spontaneous or induced tumors (16, 70, 154, 271).

More recently, these observations have been extended to the Mps1 kinase. The human Mps1 gene contains several mononucleotide repeats (A9 and A7 repeats in exon 5) which harbor frequent (~35%) mutations in gastric and colorectal tumors with microsatellite instability (3). All these alterations are frameshift mutations that result in premature stops of Mps1 protein synthesis. Mps1 mutations also have putative driver oncogenic potential in pancreatic cancer (46). As discussed above for Plk1 or Plk4, overexpression of these checkpoint kinases in human cancer (FIGURE 6) represents either a passenger alteration or suggest that both loss- and gain-of-function alterations in these proteins may cooperate in tumor development.

5. Other cell cycle kinases

Cdc7 is overexpressed in some tumor types (30, 97, 180), and the corresponding locus is amplified in breast tumors (65). Nek2, -6, and -8 are upregulated in several cancers (33, 123, 124), and a specific mutation of Nek8 has also been proposed to drive pancreatic cancer (46). Nek2 has been shown to mediate certain oncogenic activities of the Ras oncogenes (369), and it may contribute to aneuploidy through its regulation of Mad2 and Cdc20 (196). Nek6 induces anchorage-independent growth and has been show to display transforming potential in vitro (155, 242).

B. Therapeutic Approaches

The almost obligatory alteration of the G1/S machinery in tumors triggered an enormous interest to inhibit cell cycle kinases in cancer therapy, even before the requirements for these kinases were analyzed in vivo (208, 212). On the other hand, the essential requirements that most cells, either normal or tumoral, have for mitotic kinases such as Aurora A/B or Plk1 also led to the incorporation of these molecules to the pipeline of major pharmaceutical companies. Both efforts respond to the necessity to impair cell proliferation, even if the targeted molecules themselves are not a frequent target of tumor-associated mutations, as in the case of Aurora or Plk1. Even more paradoxically, some targets such as the checkpoint kinases are clearly tumor suppressors (upon partial inhibition) and, yet, their further inhibition is likely to impair proliferation of cancer cells. A second class of therapeutic strategies is based on the abrogation of the cell cycle checkpoints, initially proposed for selecting DNA damage response kinases for cancer therapy (164, 280). These strategies are based on the idea that inactivating the checkpoints in rapidly proliferating cells may lead to the accumulation of DNA damage and genomic instability in these tumor cells. In both cases, kinases have been selected as prime targets for these therapeutic efforts given the relative simplicity of obtaining small molecule inhibitors of the kinase activity. Finally, we will discuss new proposals to disrupt mitotic exit, thus leading to apoptotic cell death in tumor cells.

1. Inhibiting cell cycle entry or progression

The critical roles of multiple cell cycle kinases in the progression throughout the cell cycle suggest that their inhibition may arrest tumor cell proliferation. In fact, inhibition of a number of these kinases or suppression of their expression by a variety of techniques results in cell cycle arrest or apoptotic cell death in culture. Those cases in which the elimination of a cell cycle kinase resulted in no cell-cycle defects are likely to be due to the presence of multiple family members or compensatory roles by other kinases.

In the case of the large family of mammalian Cdk5s, these compensatory roles have been at least partially analyzed through the use of mouse knockout models (209, 211). Thus, although Cdk2 was initially one of the major targets for inhibiting G1/S progression in tumor cells, the discovery of its complete dispensability during mitosis in mammals (23, 251) was critical to reevaluate which of the G1 Cdk5s may be more relevant for cancer therapy. The answer is likely to depend on the cell of origin of the tumor and the genetic background that drives malignancy in these cells (214). For instance, Cdk6 is likely to be a major target for lymphoid malignancies (135), whereas Cdk4 may be a relevant target in multiple epithelial tumors (214). The best demonstration that Cdk4 is required for specific malignancies comes from the genetic elimination of this protein in several tumor types induced by specific oncogenes. The pioneer work by P. Sicinski group showed that cyclin D1 is required for Ras-but not Myc-induced breast tumors (366), and this requirement was later attributed to the cyclin D1-dependent kinase activity of Cdk4 (184, 210, 367). In the lung, genetic ablation of Cdk4, but not Cdk2 or Cdk6, induces an immediate senescence response only in oncogenic lung cells that express an endogenous K-Ras oncogene (266). This result demonstrates the relevance of Cdk4 to prevent senescence induced by oncogenes in vivo and the relevance of Cdk4 to arrest tumor development induced by Ras oncogenes. Cdk2 may play similar roles downstream of the Myc oncogene as Cdk2 ablation prevents senescence in Myc transformed cells (43, 145). These studies are likely to strongly contribute to the adequate design of therapeutic strategies aimed to inhibit Cdk4 in the right tumor cell, i.e., proper selection of the tumor type and the initiating oncogenic alterations (214). Other therapeutic efforts to inhibit Cdk2, Cdk1, or Cdk5 have also resulted in promising data in different preclinical assays (81, 91, 110), although the identity of the relevant target in these studies is in most cases compromised due
to the lack of specific compounds or the use of in vitro systems. Similarly, transcriptional Cdks are also considered as putative cancer targets, although no data are available on the recommended therapeutic uses of these strategies (41, 347).

Cdc7 cooperates with Cdks in the regulation of DNA replication from licensed chromosomes and is completely required for efficient S-phase progression (302). Multiple recent studies have led to the proposal of Cdc7 as a new important cancer target. Inhibition of Cdc7 kinase activity results in a decrease of Mcm2 phosphorylation, restricts DNA replication, and induces apoptotic cell death (60, 234). Several Cdc7 inhibitors have been tested in preclinical studies, and two of these inhibitors are currently in phase I clinical development (233, 318).

Several small molecules targeting Aurora kinases A and B or Plk1 have been evaluated preclinically and in early-phase trials (31, 259). Inhibition of Aurora activity leads to failure in chromosome segregation, accumulation of tetraploid cells, and ultimately apoptosis, preferentially in cells with compromised p53 function (279). Several Aurora inhibitors have been tested in clinical trials so far. Most of them inhibit all Aurora kinases as well as other kinases such as Abl, Ret, Flt3, or Jak kinases. Neutropenia is the most common toxicity, and some partial responses and stable disease have been described, although it is currently impossible to discriminate whether the effective targets are Aurora kinases or some other leukemia targets such as Abl (31). Aurora A-specific inhibitors (MLN8054 and MLN8237; Millennium Pharmaceuticals) have also shown partial stabilization of the disease in phase I clinical trials and need to be further analyzed in new trials. The Aurora B-specific inhibitor AZD1152 (AstraZeneca) also showed stabilization of the disease in <50% of patients (31). Most of these treatments resulted in neutropenia, a dose-limiting side effect of several anticancer drugs. Inhibition of Aurora kinases does not result in thrombocytopenia, probably as a consequence of the natural downregulation of these kinases and subsequent polyploidization during maturation of megakaryocytes (165). In general, it is too early to evaluate the advantage of using these drugs, and further studies are required. Indeed, a plethora of additional Aurora inhibitors have demonstrated antitumoral effects in preclinical assays but have not entered clinical trials yet.

Several Plk1 inhibitors (BI-2536, GSK-461364, ON-01910, and HMN-214) have been studied in clinical trials, and some other drugs are in development (75, 296). In general, these inhibitors showed partial antitumor activity in the first clinical trials enrolling patients with advanced solid tumors and refractory or relapsed acute myeloid leukemia. Adverse events included neutropenia and other hematologic toxicities narrowing the therapeutic index in these trials (75, 238, 297, 348). In the case of Plks, additional efforts are ongoing to identify and validate small molecules that bind to the PBD to inhibit the interaction of Plk1 with its partners (259). All these efforts, however, must take into account that inhibition of Plk2, Plk3, Plk4, or Plk5 may lead to tumor development as these less-known Plks may function as tumor suppressors in specific cell types as described above.

Only preliminary in vitro studies are available for other cell cycle kinases due to the lack of specific compounds or in vivo models. Nek kinases are putative cancer targets as suggested from multiple in vitro data (124, 177, 242, 331), and the first efforts to identify specific inhibitors have been recently reported (125). The discovery of a new molecular mechanism of regulation of Nek2, Nek6, and Nek7 kinases (274) has also opened the way for the search of specific inhibitors of these proteins that do not target other kinases.

Recent data also suggest that Mastl, the mitotic kinase that inhibits PP2A (38, 48, 341), may also be a relevant target for anticancer therapy. Inactivation of Mastl would be predicted to activate PP2A, a well-known tumor suppressor (82), and to inhibit mitotic progression in dividing cells (38). Furthermore, the combination of Mastl inhibitors with Cdk1 inhibitors may be of particular efficacy in preventing the division of cancer cells by lowering Cdk1 activity and increasing the activity of the PP2A that remove Cdk-dependent phosphates in critical cell cycle substrates.

2. Checkpoint abrogation

The primary function of the SAC is to prevent chromosome missegregation and genomic instability. As described above, several checkpoint proteins are mutated in human cancer, usually as heterozygous, partial loss-of-function mutations. These abnormalities may eventually participate in malignant transformation by allowing chromosomal instability and the selection of malignant chromosome arrangements. However, further disruption of the mitotic checkpoint may also confer therapeutic advantages by causing aberrant divisions and apoptotic cell death in tumor cells. In fact, in the absence of a functional checkpoint, as occurs when Bub1 or Mps1 function is lost, cells become rapidly aneuploid and subsequently die (152, 178). Thus elevating the frequency of chromosome missegregation may be adopted as a strategy to kill tumor cells. This approach and the strong requirements for Mps1 during the cell cycle have led to an intense search for small molecule inhibitors for this kinase (66, 67, 80, 183, 287, 295). Inhibiting these mitotic kinases is also likely to synergize with spindle damaging agents such as taxanes or vinca alkaloids as cells with decreased levels of SAC proteins not only will experience defective microtubule-kinetochore attachments.
but will also be unable to sense the errors, being thereby highly prone to genetic instability. The clinical impact of these proposals remains to be tested, but it is likely to promote an intense research in the field in the next few years.

3. Inhibiting cell cycle exit

Many therapeutic strategies aimed to inhibit the cell cycle result in arrested tumor cells that may eventually reenter the cell cycle. A new therapeutic strategy has been suggested in which tumor cells are allowed to progress throughout the cell cycle, but mitotic exit is prevented by impairing the APC/C-Cdc20-dependent degradation of cyclin B (137). Thus elimination of Cdc20 by RNAi or genetic ablation results in a dramatic metaphase arrest that is followed by p53-independent apoptotic cell death in every cell tested (137, 217). In fact, genetic ablation of Cdc20 results in a dramatic elimination of epithelial or mesenchymal tumors in vivo, whereas similar treatments with other mitotic drugs (microtubule poisons or Plk inhibitors) only result in partial arrest of tumor growth (217). Although this strategy requires further refinement to discriminate between normal and tumor cells, it may provide therapeutic advantages when putative APC/C-Cdc20 inhibitors are properly delivered or in combination with other therapies (e.g., microtubule poisons such as taxol) whose efficiency correlates with the length of mitosis. Although the molecular pathways involved in mammalian exit are not well understood, our recent work supports the relevance of inhibiting Mastl to allow PP2A activity during mitotic exit. In fact, inhibiting Cdk1 and/or Mastl may prevent cell cycle arrest and apoptosis after Cdc20 ablation (217). Thus inhibiting these two kinases may actually counteract the therapeutic effect of inhibiting APC/C-Cdc20 in tumor cells.

VI. SUMMARY AND FUTURE PERSPECTIVES

The intense research on the regulation of the cell division cycle has led to the discovery of a significant number of genes and molecular complexes involved in cell cycle regulation (143, 243). For instance, ~600 human genes result in mitotic phenotypes when inhibited by RNAi (243), and the number of proteins potentially affecting G1/S regulation (e.g., mitogenic pathways) is much higher. As an example, 7,161 gene products are classified as “cell cycle” molecules in the Gene Ontology database (GO:0007049; http://www.geneontology.org/). Similarly, multiple protein kinases are likely to affect cell cycle progression at different levels. In this review, we have selected those kinases whose main function is to regulate the cell cycle by direct phosphorylation of critical components of the cell cycle machinery.

As a first conclusion from this review, three major groups of cell cycle kinases can be established based on their function, cellular requirements, and even therapeutic use: 1) kinases required for cell cycle entry, 2) kinases required for cell cycle progression, and 3) checkpoint kinases (FIGURE 7).

Cell cycle entry kinases (e.g., Cdk4/6) are typically inhibited in quiescent cells, and their reactivation is responsible for entry into the cell cycle in response to proper mitogenic signals. These kinases are not required for the basic cell cycle, for instance, in embryonic cells as these cells express all the other regulators required for cell cycle progression but do not express the repression machinery required for differentiation. Thus, as suggested by Boveri more than a century ago (32), cell division is part of the nature of the cells, and the repression/derepression machinery is only required for regulating this intrinsic proliferative potential of cells. Therefore, these kinases are likely to be crucial regu-

FIGURE 7 Essential roles and therapeutic uses of cell cycle kinases in human cancer. Inhibition of cell-cycle-entry kinases may arrest tumor cells in G0/G1 without major damage, although apoptosis is observed in some cases, probably as a consequence of the survival function of E2F factors. Inhibition of core kinases should prevent cell cycle progression as long as all compensatory or related kinases are inhibited. This arrest is likely to lead to aberrant ploidy or apoptosis as these cells already started S phase. Checkpoint abrogation should lead to genomic instability and apoptosis upon continuous treatments. Other kinases or pathways not discussed in the main text (such as DNA damage) are not considered here.
lators of stem cell function; regenerative potential of tissues and its deregulation is a hallmark alteration in human cancer.

The core kinases required for the cell division cycle (e.g., Cdk1, Cdc7, Aurora A/B, or Plk1) are well conserved throughout evolution and are essential for cell cycle progression. Nonessential phenotypes are only observed by compensation by other family members that have been generated throughout evolution. The expression of these kinases is altered in some tumors, although it is not clear to what extent this alteration reflects proliferative potential. Its inhibition is likely to be lethal for every cell, although the increased proliferative potential of tumor cells, as well as other strategies that we still need to delineate (e.g., combination treatments), may allow their use as therapeutic targets.

Checkpoint kinases (Bub1, BubR1, and Mps1) are not strictly required for cell cycle progression but are critical to avoid errors and to prevent genomic instability. In most studies, the inhibition of these kinases results in lethal phenotypes, although they are not usually found within the first cell cycle, and they may be a consequence of the inefficient chromosome alignment in human cultured cells. Their inhibition may be used for therapeutic strategies aimed either to inhibit cell cycle progression or to promote massive instability in tumor cells by abrogating the corresponding checkpoints.

One aspect not covered in the previous classification is the cell-type specificity of some of these kinases. Thus it was initially surprising that Cdk5 function mostly affects neurons or that Cdk4-null mice are diabetic and Cdk2-null mice are sterile, despite the absence of alterations in any other tissue (209). In addition, the complexity of the different cell cycles in multicellular organisms requires a different combination of kinases to coordinate cellular responses with the rest of the cells in the tissue or with other tissues. On one hand, the variety of mitogenic signals may require a wide panel of cyclins (~25 in the human genome) and Cdk5 (20 Cdns in humans; Ref. 211) to control reentry into the cell cycle. On the other hand, different variants of the cell cycle (endoreduplication, acytokinetic, or even meiosis) require specific machineries or specific regulation of the existing enzymes. As discussed above, we now have a preliminary picture of the physiological relevance of many of these kinases in mammals.

Given that kinases are druggable targets, the physiological relevance of these proteins is a major issue in human disease. Cell cycle kinases are promising therapeutic targets for multiple diseases, and their inhibition may be beneficial in neurodegenerative diseases (Parkinson’s and Alzheimer’s diseases; Cdk5), cardiac failure and hypertrophic cardiomyopathy (Cdk9), and cancer (multiple Cdns, Cdc7, Auror, Plk1, Nek kinases etc.). On the other hand, reexpression or activation of cell cycle kinases (e.g., Cdk4 or Cdk2) may be used to improve the regenerative potential of some organs such as the heart after cardiac injury or in specific hypomorphic syndromes such as hypopituitarism. In any case, the balance between the therapeutic benefit of modulating these activities and the undesirable side effects remains as a major issue to be solved. Research efforts in the next years are likely to improve this picture by adding new kinases, providing a better understanding of their molecular function and their tissue-specific requirements, and finding synthetic lethal interactions of clinical relevance.

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