To ensure proper progression through the cell cycle, cells have developed a series of checkpoints that prevent them from entering into a new phase of the cycle until they have successfully completed the previous one. Recently divided or quiescent cells also must pass certain checkpoints before they enter a new cycle. For instance, these cells must determine whether they have all the necessary nutrients to carry out cell division. They also need to sense that they have reached proper homeostatic cell size, otherwise they will decrease (or increase) in size with each round of division. Metazoans must also control the number of cells in every organ, a parameter that, coupled with cell size, ultimately determines the size of every individual. In addition, cells must control the amount of mitogenic information required to enter the cycle (for review, see Malumbres and Barbacid 2001). Too stringent requirements would prevent cell proliferation at critical times such as during wound healing or to fight an infection. On the other hand, overly relaxed controls would lead to unscheduled proliferation and possibly neoplastic growth.

The mammalian cell cycle (see Fig. 1) is thought to be driven by heterodimeric kinases composed of a catalytic subunit known as cyclin-dependent kinase (Cdk) and a regulatory subunit, designated as cyclin. Two of these Cdks, Cdk4 and Cdk6, bind to and are activated by the D-type family of cyclins, D1, D2, and D3 (for review, see Sherr and Roberts 1999). The D-type cyclins are critical integrators of mitogenic signaling since their synthesis is one of the main endpoints of the Ras/Raf/MAPK signaling pathway. Interestingly, mouse embryonic fibroblasts that lack the three D-type cyclins can proliferate, indicating that these molecules are not strictly necessary for cell cycle progression at critical times such as during wound healing or to fight an infection. On the other hand, overly relaxed controls would lead to unscheduled proliferation and possibly neoplastic growth.

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The primary role of the D-type cyclins/Cdk4 or Cdk6 complexes is to phosphorylate members of the retinoblastoma (Rb) protein family, pRb, p107, and p130. The Rb proteins are tumor suppressors that in their active, non-phosphorylated state prevent expression of genes necessary for DNA replication (S phase of the cell cycle) and mitosis (M phase). Phosphorylation of pRb by D-type cyclins/Cdk4 or Cdk6 complexes results in its partial inactivation, which, in turn, allows expression of a limited number of transcriptional targets needed to drive cells through the G1 phase of the cell cycle (for review, see Co...
Some of these targets include the E-type cyclins (E1 and E2), whose primary role is to activate Cdk2. Active E-type cyclin/Cdk2 complexes further phosphorylate pRb, leading to a wave of transcriptional activity essential to proceed through the G1/S transition.

Cdk activity can be negatively regulated by Cdk inhibitors (CKI). CKIs come in two flavors. The four-member INK4 family (p16INK4a, p15INK4b, p18INK4c, and p19INK4d) exerts its inhibitory activity by binding to the Cdk4 and Cdk6 kinases and preventing their association with D-type cyclins (Sherr and Roberts 1999). Although INK4 proteins are biochemically indistinguishable from each other in vitro, they are expressed at different times during embryonic and postnatal development. Moreover, generation of gene-targeted mice deficient for each of these proteins has revealed significant functional differences for review, see Ortega et al. (2003). Although most of these tumor-associated alterations result from DNA mutations, it is becoming increasingly evident that epigenetic silencing of certain promoters (e.g., p16INK4a, p15INK4b, or p19INK4d) also plays a significant role in tumor development (for review, see Esteller 2005).

Direct genetic or epigenetic alteration of Cdk2 has been rarely described. Yet, expression of some of its most direct regulators, p27Kip1 and cyclin E1, is frequently altered in human tumors, changes that often correlate with poor prognosis (for review, see Ye et al. 2001). Within the Rb protein family, loss or inactivation of pRb is a rather frequent event in human tumors. However, p130 is less frequently lost, and p107 inactivation has not been reported (for review, see Esteller 2005.

For related information on mice deficient in the corresponding cyclins, see Ciencinska and Vojnov (2005).

**Table 1. Genetic Analysis of Mammalian Cell Cycle Cdks**

<table>
<thead>
<tr>
<th>Cdk</th>
<th>Alteration</th>
<th>Viability</th>
<th>Phenotype in vivo</th>
<th>Phenotype in vitro</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cdk4</td>
<td>R24C mutant insensitive to INK4 inhibitors</td>
<td>viable</td>
<td>epithelial and mesenchymal tumors with complete penetrance after 14-18 months</td>
<td>faster cell cycles and no &quot;culture crisis&quot;; increased susceptibility to transformation by Ras oncogenes</td>
<td>Stoltz et al. (2001a,b)</td>
</tr>
<tr>
<td>Cdk4</td>
<td>null mutation</td>
<td>viable</td>
<td>lack of proliferation of postnatal pancreatic β-cells and jejunitary lactoctyph, small size</td>
<td>decreased susceptibility to immortalization or transformation by oncogenes</td>
<td>Rane et al. (1999); Tsutsumi et al. (1999); Moore et al. (2002); Marin et al. (2003)</td>
</tr>
<tr>
<td>Cdk6</td>
<td>null mutation</td>
<td>viable</td>
<td>defective erythroid lineage development</td>
<td>no phenotype in MEFs but delayed proliferation of lymphoid cells</td>
<td>Malumbres et al. (2004)</td>
</tr>
<tr>
<td>Cdk4 and Cdk6</td>
<td>double null mutant</td>
<td>late embryonic lethality</td>
<td>limited proliferation of committed hematopoietic precursors, especially those of erythroid origin</td>
<td>delayed cell cycles and decreased pRb phosphorylation; cells respond to mitogenic stimuli and become immortal upon passage</td>
<td>Malumbres et al. (2004)</td>
</tr>
<tr>
<td>Cdk2</td>
<td>null mutation</td>
<td>viable</td>
<td>male and female sterility due to meiotic defects; no defects in mitotic cells</td>
<td>Early senescence in MEFs in culture; no major cell-cycle defects</td>
<td>Ortega et al. (2003)</td>
</tr>
<tr>
<td>Cdk2</td>
<td>conditional null mutation</td>
<td>viable</td>
<td>no phenotype observed</td>
<td>no defects in cell proliferation after ablation</td>
<td>our unpublished observations</td>
</tr>
<tr>
<td>Cdk2 and Cdk6</td>
<td>double null mutation</td>
<td>phenotype identical to Cdk2 and Cdk6 single mutants</td>
<td>phenotype identical to Cdk2 and Cdk6 single mutants; no synergism</td>
<td>Malumbres et al. (2004)</td>
<td></td>
</tr>
<tr>
<td>Cdk3</td>
<td>premature stop</td>
<td>viable</td>
<td>normal; most laboratory strains carry this mutation</td>
<td>normal; mutation present in &quot;wild type&quot; MEFs</td>
<td>Ye et al. (2001)</td>
</tr>
</tbody>
</table>

For related information on mice defective in the corresponding cyclins, see Ciencia and Vojnov (2005).
GENETIC ANALYSIS OF CDKS

WIDESPREAD TUMOR INDUCTION IN MICE EXPRESSING A MISREGULATED Cdk4 KINASE

Most human tumors have genetic and/or epigenetic alterations in the cyclin D-INK4-Cdk4-Cdk6 pathway. As indicated above, a miscoding mutation (replacement of Arg24 by Cys) that renders Cdk4 resistant to INK4 inhibitors is associated with the development of human melanoma. To study the effects of such mutation in the regulation of the cell cycle in particular and in tumorigenesis in general, we have engineered knockin mice that carried this mutation within the endogenous mouse Cdk4 locus. (Sotillo et al. 1999) Cdk4R24C/R24C mice are born at the expected Mendelian ratio, are fertile, and develop normally. However, these mice developed detectable tumors after 8 months and most of them died by 16 months of age (Sotillo et al. 2001a). Necropsy of more than 100 Cdk4R24C/R24C mice showed a wide spectrum of tumors, including malignancies of mesenchymatous origin (67% incidence), epithelial endocrine (55%), and, to a lesser extent, hematopoietic malignancies (3%). Many mice exhibit multiple tumors of independent origin, with an average of almost two tumors per animal. A cohort of 50 mice sacrificed at 14–16 months of age without external signs of disease revealed a similar tumor distribution pattern, except for reduced incidence of sarcomas and pituitary tumors, suggesting that these malignancies are a major cause of death in Cdk4R24C/R24C mice. Cdk4R24C mice also succumbed to the same types of malignancies, although with increased latency (Sotillo et al. 2001a). Cdk4 misregulation synergizes with mutations in other cancer genes such as p53. Double mutant Cdk4R24C/R24C p53−/− mice die before reaching 4 months of age, significantly earlier than their Cdk4R24C p53−/− littermates (Sotillo et al. 2001a). These mice display an increased number of sarcomas, mainly hematiosarcomas and leiomyosarcomas. Moreover, a significant fraction of these double mutant mice (10%) develop immature teratomas, a tumor not found in any of their single mutant siblings, although a low incidence of teratomas (<2%) has been described previously in p53−/− mice. These results suggest that these key regulators exert growth control at different threshold levels in different cell types.

A MUTANT Cdk4R24C KINASE INDUCES INVASIVE MELANOMAS IN MICE

Interestingly, Cdk4R24C/R24C mice do not develop melanomas, the type of tumor induced by this very same mutation in humans. To determine whether these observations were a consequence of intrinsic mechanistic differences between human and rodents or of phenotypic differences, we submitted the skin of these mutant mice to carcinogenic insults. Specifically, Cdk4R24C/R24C mice were treated with 7,12-dimethylbenz[a]anthracene (DMBA) followed by repeated exposure to 12-O-tetradecanoyl-phorbol-13-acetate (TPA), a treatment known to induce the rapid development of skin papillomas. Unlike wild-type mice, Cdk4R24C/R24C animals develop a high number of nevi that rapidly grow in size, leading to the formation of tumors of up to 20 mm in diameter by 20 weeks (Sotillo et al. 2001b). Alternative treatments with DMBA plus UVB light also result in the development of similar tumors, albeit with reduced efficiency. No melanocytic tumors are observed after single treatments with either DMBA or UVB light alone. Histological examination of these lesions confirmed the presence of melanomas in 70% of carcinogen-treated Cdk4R24C/R24C mice (Sotillo et al. 2001b). Unlike other models of melanoma, these tumors are highly melanotic, a property that makes them more reminiscent of those observed in humans. Moreover, as observed in human melanomas, the percentage of melanotic cells decreases in advanced malignancies, since active proliferation and melanocyte dedifferentiation often result in decreased production of melanin. In the more advanced melanomas, Cdk4R24C/R24C tumors are highly cellular, mainly composed of proliferating atypical melanocytes, frequently spindle-shaped with low or no load of melanin. Mitotic figures and areas of necrosis can be easily found. In these cases, melanocyte proliferation is usually invasive to neighbor tissues with infiltrating margins. The neuroectodermal origin of the melanomas is confirmed by positive immunological staining for the S100 antigen. Molecular analysis of Cdk4R24C/R24C melanomas did not reveal either deletion, rearrangement or promoter methylation in p16Ink4a, p19Arf, p53, or p19Arf loci (Sotillo et al. 2001b). Although these results are expected for those genes encoding upstream INK4 regulators, the absence of p19Arf inactivation suggests that alterations in the p53 pathway are not needed for induction and/or progression of these melanomas.

GENETIC ANALYSIS OF THE ROLE OF Cdks IN VIVO

Targeting the cell cycle is an attractive strategy to block neoplastic growth, since most human tumors carry molecular alterations in genes that regulate cell cycle commitment. Unfortunately, most cell cycle regulators altered in human cancer, such as loss of p27kip1, p16Ink4a, or p19Arf expression, overexpression of cyclin D and cyclin E, etc., are not amenable to classic pharmacological approaches. On the other hand, the Cdks, regardless of whether they are mutated or not, are suitable targets for therapeutic intervention. Indeed, kinase inhibition has already been successfully used for therapeutic purposes in many diseases, including cancer (e.g., Gleevec). Yet, our current knowledge of the cell cycle is not sufficient to ensure the development of optimal strategies. For instance, we do not know which Cdk inhibitors will provide the best therapeutic benefit or whether it would be more efficacious to block two or several Cdks at the same time.

To gain further knowledge about the role of individual Cdks in the proliferation of normal and tumor cells, we have embarked on the systematic analysis of each of the four cell cycle Cdks: Cdk1, Cdk2, Cdk4, and Cdk6, at the genetic level using gene-targeted strategies in embryonic stem cells. A fifth Cdk also implicated in the cell cycle
β expression is basically undetectable in postnatal pancreatic epithelial cells (Martín et al. 2003). Interestingly, Cdk6 expression is also undetectable in postnatal pancreatic cells (Rane et al. 1999; Tsutsui et al. 1999). Yet, these mice have limited populations of certain endocrine cell types. For instance, adult Cdk6 null mice become diabetic due to reduced numbers of insulin-producing pancreatic β-cells (Rane et al. 1999; Tsutsui et al. 1999). This phenotype is not a consequence of the hormonal deficiencies in the bone marrow. In vitro, Cdk6 null MEFs also exhibit lower numbers of red cells (about 15%) in peripheral blood. Interestingly, Cdk6−/− mice do not display obvious deficiencies in the bone marrow. In vivo, Cdk6 null lymphocytes, but not Cdk6−/− MEFS, show delayed entry in the cell cycle upon mitogenic stimulation (Malumbres et al. 2004).


cdk4 null MICE

Ablation of Cdk4 does not have significant consequences for cell proliferation, at least in mouse embryonic fibroblasts (MEFs). Quiescent Cdk4 null (Cdk4−/−) MEFs enter S phase with slightly delayed kinetics but proliferate normally (Rane et al. 1999; Tsutsui et al. 1999). In vivo, Cdk4 null mice are viable, indicating that Cdk4 is not essential for proliferation of most cell types (Rane et al. 1999; Tsutsui et al. 1999). Yet, these mice have limited populations of certain endocrine cell types. For instance, adult Cdk4−/− mice become diabetic due to reduced numbers of insulin-producing pancreatic β-cells (Rane et al. 1999; Tsutsui et al. 1999). Subsequent studies have indicated that Cdk4 is essential for postnatal proliferation of pancreatic β-cells but not for their genesis from ductal epithelial cells (Martin et al. 2003). Interestingly, Cdk6 expression is basically undetectable in postnatal pancreatic β-cells, thus providing a possible explanation for their strict dependence on Cdk4 expression. Cdk4 null mice also have decreased male fertility due to defective spermatogenesis and reduced numbers of Leydig cells (Rane et al. 1999; Tsutsui et al. 1999). Female Cdk4−/− mice are completely sterile due to limited prolactin production, a consequence of their reduced numbers of pituitary lactotrophs (Moons et al. 2002; Jirawatnotai et al. 2004). As in the case of pancreatic β-cells, Cdk4 is essential for postnatal proliferation of the anterior pituitary, but it is not required for embryonal development of the pituitary gland (Moons et al. 2002). Interestingly, siRNA-mediated knockdown of Cdk4, but not of Cdk6, inhibits GH/RH-induced proliferation of GH3 somato/lactotroph cells with restored expression of GHRH receptors, thus suggesting distinct functional roles for these highly related kinases within the same cellular context (Jirawatnotai et al. 2004).

Finally, Cdk4−/− mice are considerably smaller than their wild-type littermates (Rane et al. 1999; Tsutsui et al. 1999). This phenotype is not a consequence of the hormonal defects of Cdk4 null animals. Cdk4−/−Rip-Cre mice, in which an active Cdk4 kinase is re-expressed in pancreatic β-cells and in pituitary lactotrophs, are normoglycemic and fully fertile, yet they retain their small-size phenotype (Martin et al. 2003). Since the size of Cdk4 null cells appears to be similar to that of wild-type cells, it is possible that Cdk4 plays a role in controlling homeotic cell numbers, at least in mice.

cdk6 null MICE

Mice lacking Cdk6 develop normally and are viable, although hematopoiesis is slightly impaired (Malumbres et al. 2004). This is a somewhat expected result, since Cdk6 is most abundantly expressed in lymphoid organs. In Cdk6-deficient mice, the thymus is reduced in size due to lower cellularity, and its cortical area is atrophic in about one-third of the mutant animals. These spleens are also reduced in size due to decreased cell density in the red pulp. Whereas in wild-type mice, 70–75% of all spleen cells belong to the erythrocyte lineage, these figures decrease to 35–40% in Cdk6−/− littermates. Similarly, the number of megakaryocytes is severely reduced to less than one-third of those present in wild-type spleens (Malumbres et al. 2004). Cdk6 null mice also exhibit limited numbers of red cells (about 15%) in peripheral blood. Interestingly, Cdk6−/− mice do not display obvious deficiencies in the bone marrow. In vitro, Cdk6 null lymphocytes, but not Cdk6−/− MEFS, show delayed entry in the cell cycle upon mitogenic stimulation (Malumbres et al. 2004).

LATE EMBRYONIC LETHALITY OF Cdk4/Cdk6 DOUBLE MUTANT MICE

Mice lacking both cyclin D-dependent kinases, Cdk4 and Cdk6, are not viable. Yet, Cdk4−/−;Cdk6−/− double mutant embryos develop to midgestation with the expected Mendelian frequency, suggesting that they do not have intrinsic cell cycle defects (Malumbres et al. 2004). This is a somewhat expected result, since Cdk6 is most abundantly expressed in lymphoid organs. In Cdk6-deficient mice, the thymus is reduced in size due to lower cellularity, and its cortical area is atrophic in about one-third of the mutant animals. These spleens are also reduced in size due to decreased cell density in the red pulp. Whereas in wild-type mice, 70–75% of all spleen cells belong to the erythrocyte lineage, these figures decrease to 35–40% in Cdk6−/− littermates. Similarly, the number of megakaryocytes is severely reduced to less than one-third of those present in wild-type spleens (Malumbres et al. 2004). Cdk6 null mice also exhibit lower numbers of red cells (about 15%) in peripheral blood. Interestingly, Cdk6−/− mice do not display obvious deficiencies in the bone marrow. In vitro, Cdk6 null lymphocytes, but not Cdk6−/− MEFS, show delayed entry in the cell cycle upon mitogenic stimulation (Malumbres et al. 2004).

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n/n; nases phosphorylate pRb, at least in vitro. Moreover, (Malumbres et al. 2004). Indeed, these Cdk2/cyclin D ki-
cognate partners, form functional complexes with Cdk2 of D-type cyclins. These cyclins, in the absence of their
kinases are not essential either for cell proliferation or for

taken together, indicate that the D-type cyclin-dependent

massive apoptosis observed in

triggers the pachytene checkpoint responsible for the

ical markers firmly established that the absence of Cdk2

totene, zygotene, and pachytene stages, but no diplotene

adult mice by immunoconfocal microscopy shows lep-

animals do not have round spermatids in the seminiferous
tubules, and there is massive apoptosis of spermatocytes

for up to 1.5 years without developing obvious additional

ABSENCE OF SYNERGISM BETWEEN Cdk6

AND Cdk2 DEFICIENCIES

Crossovers between Cdk6−/−, Cdk2−/− heterozygous mice yield Cdk6−/−;Cdk2−/− double null animals with expected
Mendelian ratios. Mice deficient for Cdk6 and Cdk2 display those phenotypical abnormalities previously observed in Cdk6 and Cdk2 single mutant strains (Ortega et al. 2003; Malumbres et al. 2004). Briefly, Cdk6−/−, Cdk2−/− animals are sterile and show marked de-

fects in spermatogenesis and oogenesis, basically indistin-
guishable from those reported for Cdk2−/− mice (Ortega et al. 2003). They also have limited defects in hematopoietic cells similar to those described above for

Cdk6 null mice. Finally, Cdk6−/−, Cdk2−/− animals survive

up to 1.5 years without developing obvious additional

abnormalities (Malumbres et al. 2004).

Cdk2 IS ALSO DISPENSABLE FOR

CELL CYCLE REGULATION MEDITATED BY

Cip/Kip INHIBITORS

The above results challenge the central roles attributed to Cdk2 in cell cycle progression. One such role involves

mediating the inhibitory properties and tumor-suppres-

Cdk4 and Cdk6 DEFECTIVE MEFs

Early passage Cdk4−/−,Cdk6−/− MEFs proliferate well, albeit at a slower rate than control cells (Malumbres et al. 2004). Moreover, all Cdk4−/−,Cdk6−/− double mutant

MEFs can immortalize upon continuous passage in cul-
ture. These MEFs also proliferate in low serum condi-
tions and respond to mitogenic stimuli induced by addi-
tion of EGF and IGF-1. Moreover, they enter S phase

upon mitogenic stimulation with normal kinetics, albeit

less efficiently than wild-type cells. These observations,
taken together, indicate that the D-type cyclin-dependent

kineses are not essential either for cell proliferation or for

re-entry in the cell cycle, at least in MEFs.

Ablation of Cdk4 and Cdk6 does not affect expression of

D-type cyclins. These cyclins, in the absence of their
cognate partners, form functional complexes with Cdk2

(Malumbres et al. 2004). Indeed, these Cdk2/cyclin D ki-
nases phosphorylate pRb, at least in vitro. Moreover, Cdk2

shRNA significantly decreases the rate of BrdU incor-

poration in Cdk4−/−,Cdk6−/− MEFs. These observa-

tions suggest that in the absence of Cdk4 and Cdk6,

Cdk2, in association with D-type cyclins, may help to

drive cells through the early phases of the cell cycle. Yet,

confirmation of this hypothesis must await genetic scruti-
nity; for instance, by generating Cdk4/Cdk6/Cdk2

triple mutant mice.

ABLATION OF THE Cdk2 LOCUS REVEALS

Cdk2 AS AN ESSENTIAL MEIOTIC KINASE

Cdk2−/− mice are born at the expected Mendelian ratio,

indicating that Cdk2 is not essential for cell proliferation in

most, if not all, tissues (Berthet et al. 2003; Ortega et al.

2003). Moreover, these mutant mice do not display
gross anatomical or behavioral abnormalities for up to 2

years of life except for severe atrophy in their gonads that

results in complete sterility. Spermatogenesis defects in

Cdk2−/− male mice become visible at P20, a time when germ

cells have completed meiosis I. P20 Cdk2−/− animals do not have round spermatids in the seminiferous
tubules, and there is massive apoptosis of spermatocytes

(Ortega et al. 2003). Analysis of spermatocytes from

adult mice by immunoconfocal microscopy shows lept-

totene, cytotene, and pachytene stages, but no diplotene

or later meiotic stages. Studies using immunohistochem-

ical markers firmly established that the absence of Cdk2

triggers the pachytene checkpoint responsible for the

massive apoptosis observed in Cdk2−/− spermatocytes

(Ortega et al. 2003).

Cdk2−/− oocytes develop normally through the lep-
totene, cytotene, and pachytene stages (E14.5-E18.5).

However, when oocytes reach the diplotene stage (P1–P2),

Cdk2 mutant oocytes display multiple defects (deoxy-
napped fibers, randomly distributed centromeres, etc.)

which indicate that Cdk2−/− oocytes also have a defect in prophase I, albeit at a later developmental stage (Ortega et al. 2003). These observations establish that Cdk2 is es-

sential for completion of the first meiotic division in both

male and female germ cells.

Interestingly, mice deficient for both E-type cyclins are

not viable, most likely due to a defect in the endoreplica-
tion of placental trophoblast giant cells (Gong et al. 2003;

Parisi et al. 2003). These observations provide genetic ev-

idence for a differential role of Cdk2 and E-type cyclins,

at least during endoreplication. This suggests that E-type

cyclins must have a partner(s) other than Cdk2. Whether

such partners are Cdk4, and whether they are functional in
cells other than trophoblasts, remain to be determined.

Cdk2 IS DISPENSABLE FOR THE MITOTIC

CELL CYCLE

Primary Cdk2−/− MEFs grow well in culture and prolif-

erate with kinetics similar to those of wild-type cells

(Berthet et al. 2003; Ortega et al. 2003). Serum-starved

Cdk2−/− MEFs enter S phase with the same kinetics as

their wild-type counterparts upon serum stimulation.

Cdk2−/− MEFs also become immortalized upon continu-

ous passage in culture. Indeed, we have only observed

subtle differences in the timing at which these cells enter
culture crisis. Moreover, removal of Cdk2 from Cdk2−/−

MEFs does not have significant consequences in their

proliferative properties, indicating that Cdk2 is dispens-

able for cell proliferation in conditions in which plastici-

ty is unlikely to play a role (Ortega et al. 2003). E-type

cyclin-deficient MEFs also proliferate well in culture.

However, these cells have a defect in cell cycle re-entry,

presumably due to a failure in loading MCM proteins

onto DNA replication origins (Gong et al. 2003). These

observations provide additional genetic evidence for the

existence of E-type cyclin partners other than Cdk2.

Whether the putative cyclin E partners responsible for

this cell cycle defect are the same as those required for en-
doreplication remains to be determined.
ing activities of p21\(^{\text{Cip1}}\) and p27\(^{\text{Kip1}}\), the best character-
ized members of the Cip/Kip family of cell cycle in-
hibitors (Sherr and Roberts 1999). Infection of primary MEFs with retroviruses expressing p21\(^{\text{Cip1}}\) or p27\(^{\text{Kip1}}\) proteins halts their proliferation regardless of the pres-
ence or absence of Cdk2 (Martin et al. 2005). About half of the Cdk2\(^{-/-}\) MEFs are halted in G1. Genetic evidence argues against a compensatory role of Cdk4 and Cdk6, since p27\(^{\text{Kip1}}\) and p21\(^{\text{Cip1}}\) effectively block cell proliferation in MEFs lacking either Cdk4 and Cdk6, or the three D-type cyclins (Kozar et al. 2004; Malumbres et al. 2004). Generation of double Cdk2/Cdk4 mutant cells or cells lacking the three G1/S Cdks Cdk2, Cdk4, and Cdk6, should help to deci-
pher the functional target(s) of the Cip/Kip inhibitors. Primary p27\(^{\text{Kip1}}\)/Cdk2\(^{-/-}\) and p21\(^{\text{Cip1}}\)/Cdk2\(^{-/-}\) MEFs display increased proliferation rates similar to those of p27\(^{\text{Kip1}}\) null or p21\(^{\text{Cip1}}\) null cells (Martin et al. 2005). These observations indicate that loss of Cdk2 does not abrogate the proliferative advantage conferred by the absence of p27\(^{\text{Kip1}}\) or p21\(^{\text{Cip1}}\) tumor suppressors. More-
over, quiescent p27\(^{\text{Kip1}}\)/Cdk2\(^{-/-}\) and p21\(^{\text{Cip1}}\)/Cdk2\(^{-/-}\) cells (Martín et al. 2005) enter S phase 4-6 hours earlier than wild-type cells upon serum stimulation (Martin et al. 2005), indicating that the shortening in S phase entry cannot be mediated by constitutive activation of Cdk2.

**Cdk2 IS NOT ESSENTIAL FOR p21\(^{\text{Cip1}}\)-MEDIATED CELL CYCLE ARREST AFTER DNA DAMAGE**

p21\(^{\text{Cip1}}\) is one of the major effectors of cell cycle arrest induced following DNA damage. Double mutant p21\(^{\text{Cip1}}\)/Cdk2\(^{-/-}\) cells display the same levels of BrdU incorporation as p21\(^{\text{Cip1}}\)\(^{-/-}\)\; MEFs upon exposure to gamma radiation or to etoposide treatment. Moreover, these double mutant cells also fail to arrest in G\(_1\) after DNA damage (Martin et al. 2005). These observations provide genetic evidence against the concept that p21\(^{\text{Cip1}}\) mediates cell cycle arrest at the DNA damage checkpoint by blocking Cdk2 activity.

**THE TUMOR SUPPRESSOR ACTIVITY OF p27\(^{\text{Kip1}}\) DOES NOT REQUIRE Cdk2**

Mice defective for p27\(^{\text{Kip1}}\) are bigger in size, display organomegaly and retinal defects, and develop frequent pinitary tumours (Fero et al. 1999; Kiyokawa et al. 1996; Nakayama et al. 1996). p27\(^{\text{Kip1}}\)/Cdk2\(^{-/-}\); double mutant mice weigh, on average, 50-60% more than wild type and display widespread organomegaly similar to p27\(^{\text{Kip1}}\) null animals, except in testes and ovaries which were as hypotropic as in Cdk2\(^{-/-}\) mice (Martin et al. 2005). p27\(^{\text{Kip1}}\)/Cdk2\(^{-/-}\) mutant animals display the same retinal defects observed in p27\(^{\text{Kip1}}\) null mice and with similar penetrance (about 10%) (Kiyokawa et al. 1996; Nakayama et al. 1996). More importantly, these double mutant mice develop pinitary tumours with the same high penetrance and latency as p27\(^{\text{Kip1}}\) single mu-
tant animals (Martin et al. 2005). The pinitary tumours of p27\(^{\text{Kip1}}\)\(^{-/-}\); Cdk2\(^{-/-}\) mice display the same elevated prolif-
erative indexes and volume sizes as those observed in p27\(^{\text{Kip1}}\) null mice. These results illustrate that Cdk2 is not required to mediate the in vivo tumor suppressor ac-
tivity of p27\(^{\text{Kip1}}\).

**CONCLUSIONS**

**Cell Cycle Mutations and Cancer**

Cancer is frequently considered as a cell cycle disease. Although many cell cycle regulatory mechanisms have been studied at length in vitro, it is still unclear how they are coordinately regulated in vivo and how their misreg-
ulation leads to human cancer. Evidence accumulated over the last decade has clearly illustrated that mutation of the same cell cycle regulators known to be mutated or epigenetically altered in human cancer also leads to tu-
mor development in mice (Ortega et al. 2002). For in-
stance, pRb\(^{-/-}\) (Jack et al. 1992; Lee et al. 1992), Cdk2\(^{-/-}\)/Cdk4\(^{-/-}\)/Cdk6\(^{-/-}\) (Rane et al. 1999; Sotillo et al. 2001a), and Ink4a/Arf\(^{-/-}\) (Serrano et al. 1996) mice develop tumors with complete penetrance. Other mutations, such as those ablating the INK4 inhibitors p16\(^{\text{INK4A}}\) and p15\(^{\text{INK4B}}\), have not led to overt tumor development (Latres et al 2000; Krimpenfort et al. 2001; Shapless et al. 2001) but seem to predispose to cancer. Whether these mutations in hu-
mans only contribute to carcinogenesis in the presence of other mutations remains to be determined. Finally, am-
plication of cyclin D1 or cyclin E in mice also leads to abnormal growth, albeit not to overt tumor growth, sug-
gest that they are contributors, but not “primary drivers” of carcinogenesis.

**Cdk2s and the Regulation of the Cell Cycle**

Genetic analysis of the role of Cdk2 has provided a se-
ries of observations that are not compatible with widely accepted models for the mammalian cell cycle. Mainly, neither the D-type cyclins nor their cognate cyclin D-de-
pendent kinases, Cdk4 and Cdk6, are essential for cell proliferation or exit from quiescence in most cell types. Only cells of hematopoietic origin appear to require at least one of these D-type cyclins or their kinase partners for proper proliferation. Several issues need to be ad-
dressed before we can fully evaluate the requirements for the D-type cyclin-dependent kinases in mammalian cells. For instance, we need to determine whether they are re-
quired in adult tissues, since it could be argued that dur-
ing embryonic development most cells do not exit the cy-
cle. Alternatively, it is possible that exit from quiescence during embryogenesis might be regulated by mechanisms distinct from those that regulate cell cycle re-entry in adult cells.

It is also possible that in the absence of Cdk4 and Cdk6, cellular plasticity allows Cdk2 to interact with the D-type cyclins and to compensate for their absence. Likewise, it could be argued that the Cdk2/cyclin E kinases may func-
tionally compensate for the lack of D-cyclin-dependent
kinase activity (Geng et al. 1999). Generation of triple
Cdk4/Cdk6/Cdk2 null mice should help to resolve some of
these issues. Regardless of potential compensatory effects by other
Cdns, or even by other less related kinases, the absolute
dispensability of Cdk2 in mitotic cells in vivo is an un-
expected finding. Despite extensive bibliography linking
Cdk2 to many critical cellular processes such as DNA
replication and centrosome duplication (Sherr and Roberts
1999), available genetic evidence to date has failed to
demonstrate a role for Cdk2 in these processes. Equally
surprising have been recent genetic observations indicat-
ing that Cdk2 is not essential for mediating the cell cycle
inhibition properties of the Cip/Kip inhibitors p21Cip1 and
p27Kip1 (Alemem et al. 2005; Martin et al. 2005). Likewise,
the presence or absence of Cdk2 has no effect on the inci-
dence or latency of tumor primaries caused by loss of
p53, since this tumor suppressor controls the synthesis of
p21Cip1 (Hale et al. 1995). Although the essential targets for p21Cip1
and p27Kip1 in tumor suppressors remain to be identified, it is
likely that they block cell cycle progression, at least par-
tially, by inhibiting Cdk1. Whether inhibition of Cdk1 will
have a selective effect on tumor cells is a key issue that
should be addressed by genetic means before selective in-
hibitors are developed. In summary, our results illustrate the need to continue
interrogating the cell cycle by genetic means. An inte-
grated view from biochemical studies, experimental ani-
mal models, and the analysis of cancer mutations will un-
doubtedly help to exploit this knowledge to develop
rational therapeutic approaches with which to target those
tumors exhibiting a deregulated cell cycle.

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M. BARBACID, S. ORTEGA, R. SOTILLO, et al.

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