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Mice thrive without Cdk4 and Cdk2

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ABSTRACT

Mammalian cell division is thought to be driven by sequential activation of several Cyclin-dependent kinases (Cdk), mainly Cdk4, Cdk6, Cdk2 and Cdk1. Since mice lacking Cdk4, Cdk6 or Cdk2 are viable, it has been proposed that they play compensatory roles. We report here that mice lacking Cdk4 and Cdk2 complete embryonic development to die shortly thereafter presumably due to heart failure. However, conditional ablation of *Cdk2* in adult mice lacking *Cdk4* does not result in obvious abnormalities. Moreover, these double mutant mice recover normally after partial hepatectomy. In culture, $Cdk4^{-/-}$;$Cdk2^{-/-}$embryonic fibroblasts become immortal, display robust pRb phosphorylation and have normal S phase kinetics. These observations indicate that Cdk4 and Cdk2 are dispensable for the mammalian cell cycle and for adult homeostasis.

1. Introduction

The widely accepted model for the mammalian cell cycle involves sequential activation of related heterodimeric protein kinases, composed of a catalytic subunit, the Cyclin-dependent kinase (Cdk), and a regulatory subunit known as Cyclin (reviewed in Malumbres and Barbacid, 2005). Two of these Cdks, Cdk4 and Cdk6, are activated by the D-type Cyclins and have been implicated in the early phases of the cycle, particularly during exit from quiescence. Cdk4/6-Cyclin D heterodimeric kinases are supposed to promote re-entry into the cycle by initiating phosphorylation of the retinoblastoma (Rb) protein family, pRb, p107 and p130 (reviewed in Adams, 2001; Sherr and Roberts, 1999). Rb phosphorylation results in the liberation of transcription factors, such as members of the E2F family (reviewed in Dyson, 1998; Trimarchi and Lees, 2002), which are bound to the hypophosphorylated Rb proteins in non-proliferating cells. These transcription factors are responsible for directing the expression of a variety of genes essential for advancing cells though the S phase of the cell cycle (Adams, 2001). Two of these genes encode Cyclins E1 and E2 which specifically bind to Cdk2. Active Cdk2–Cyclin E complexes further phosphorylate the Rb protein family, resulting in their complete inactivation. This process is considered to be essential for the liberation of transcription factors that mediate the synthesis of other cell cycle regulators such as the A-type Cyclins and Cdk1. Sequential activation of Cdk2 and Cdk1 by the A-type Cyclins is believed to be

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essential for the successful duplication of the cellular genome during the S phase and for progression into mitosis (Dyson, 1998; Trimarchi and Lees, 2002).

This model, mainly deduced from biochemical evidence, has not sustained genetic scrutiny. For instance, all mouse cell types, with the exception of pancreatic beta cells and pituitary lactotrophs proliferate normally in the absence of Cdk4 (Rane et al., 1999; Tsutsui et al., 1999). Likewise, ablation of Cdk6 only results in reduction of a subset of hematopoietic cells (Malumbres et al., 2004). Loss of both of these enzymes causes a much more dramatic phenotype that limits the proliferation of hematopoietic cell lineages leading to late embryonic lethality (Malumbres et al., 2004). Yet, double mutant embryos show normal proliferation rates in other tissues, indicating that Cdk4 and Cdk6 only play compensatory roles in cells of hematopoietic lineages. In agreement with these observations, mouse embryonic fibroblasts (MEFs) lacking Cdk4 and Cdk6 proliferate well and become immortal upon continuous culture in vitro. More importantly, they exit quiescence upon mitogenic stimuli and enter S phase with normal kinetics (Malumbres et al., 2004). Similar results have been obtained in mice lacking the three D-type Cyclins (Kozar et al., 2004). Likewise, Cdk2, a kinase previously thought to be essential for driving cells through the G1/S transition, is dispensable for normal embryonic development and adult homeostasis (Berthet et al., 2003; Ortega et al., 2003). Unexpectedly, Cdk2 is essential for the first meiotic division of both male and female germ cells, an activity that cannot be compensated by any of the other Cdks (Ortega et al., 2003).

These observations have been attributed, at least in part to compensatory activities between Cdk2 and Cdk4. In this manuscript, we report the generation and characterization of mice carrying germ line as well as conditional mutations in the loci encoding these kinases. Mice lacking Cdk4 and Cdk2 in the germ line complete embryonic development and are born alive. Although they die soon thereafter possibly due to their limited numbers of cardiomyocytes, the rest of the tissues display normal levels of cell proliferation. More importantly, conditional ablation of *Cdk2* in adult *Cdk4* knock out mice does not result in detectable abnormalities even in highly proliferating tissues. Indeed, these double mutant mice efficiently regenerate their livers after partial hepatectomy (PH). These observations indicate that Cdk4 and Cdk2 are dispensable for mammalian cell division and raise further questions about their proposed role in driving the mammalian cell cycle.

2. Results

2.1. Complete embryonic development in the absence of Cdk4 and Cdk2

We have generated double mutant $Cdk4^{+/-}$;$Cdk2^{+/lox}$ and $Cdk4^{+/-}$;$Cdk2^{+/-}$ mice by targeting the *Cdk4* locus in ES cells carrying a conditional $Cdk2^{lox}$ allele (Figure 1). Intercrosses between $Cdk4^{+/-}$;$Cdk2^{+/-}$ double heterozygous animals result in the generation of midgestation (E13.5) $Cdk4^{-/-}$;$Cdk2^{-/-}$

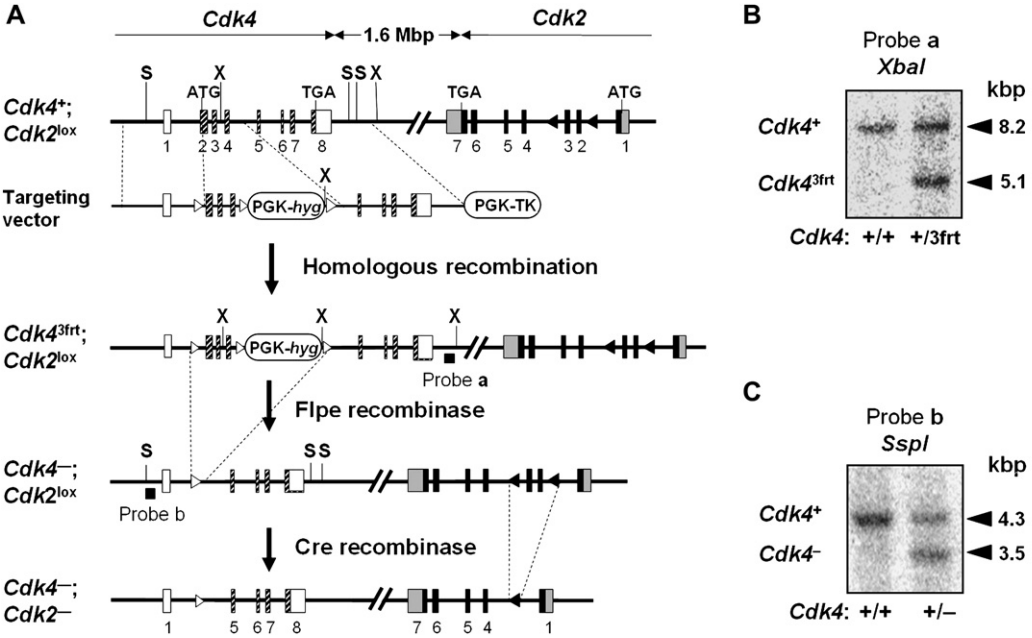

Figure 1 – Generation of $Cdk4^{-/-}$;$Cdk2^{lox/lox}$ and $Cdk4^{-/-}$;$Cdk2^{-/-}$ mice. (A) Targeting strategy. The targeting vector carries three *frt* sequences flanking exons 2 and 4 as well as a PGK-HygR cassette used for positive selection. The vector also contains a PGK-TK cassette used for negative selection. Recombinant ES cell clones containing a floxed $Cdk2^{lox}$ allele were used to generate mice carrying the $Cdk4^{3frt}$ and $Cdk2^{lox}$ alleles in the same chromosome. These mice were sequentially crossed with transgenic mice expressing the Flpe (pCAG-Flpe) and Cre (CMV-Cre) recombinases to generate heterozygous $Cdk4^{-/-}$;$Cdk2^{-/-}$ mice. *Cdk4* coding sequences are indicated by hatched boxes. *Cdk4* non-coding exons are indicated by open boxes. *Cdk2* coding sequences are indicated by black boxes. *Cdk2* non-coding exons are indicated by grey boxes. *frt* and *lox*P sites are indicated by open and closed triangles, respectively. Only the restriction sites used in these diagnostic hybridizations are indicated. The location of probes a and b used in Southern blot analysis is indicated by a thick line. (B) Southern blot analysis to identify the $Cdk4^{3frt}$ recombinant allele. (C) Southern blot analysis to identify the *Cdk4*$^{-}$ *null* allele.