Cell cycle progression requires the coordinated activation of several kinases. Some of these proteins, the cyclin-dependent kinases (Cdk) are activated upon binding of regulatory subunits, known as cyclins, that are only available during specific phases of the cycle.

At least four Cdk's –Cdk4, Cdk6, Cdk2 and Cdk1– are thought to play key roles during the different phases of the cell cycle (Figure 1). For instance, Cdk4 and Cdk6, two kinases that can only be activated by D-type cyclins, are thought to be essential in allowing cells to exit quiescence (G0) by initiating the sequential phosphorylation of the retinoblastoma protein (pRB). Partially phosphorylated pRB releases certain transcription factors such as the members of the E2F family that in turn facilitate expression of cyclin E.

Cyclin E binds to and activates its only known partner, Cdk2, to complete phosphorylation (and thus, inactivation) of pRB. pRB inactivation is a step thought to be necessary to pass the restriction point (R), a mitogenic sensor that controls commitment to initiate DNA synthesis. Loss of pRB activity sets in motion a complex transcriptional program that allows the synthesis of molecules required for the precise replication of the cellular genome. Passage through the G1/S transition results in degradation of cyclin E by the proteasome. This event allows Cdk2 to bind cyclin A until completion of the S phase. The G2/M
transition as well as mitosis is controlled by yet another kinase, Cdk1, which is sequentially activated by cyclin A and cyclin B. Cdk1/cyclin B complexes are thought to be the key regulators of mitosis.

Unveiling the molecular mechanisms that control the cell cycle is critical to understand the molecular bases of cancer. Indeed, most human tumors carry mutations that either deregulate the activity of those kinases involved in the interphase of the cell cycle. They include mutations in regulators of the G0/G1 transition thought to be controlled by Cdk4 and Cdk6 and in those that control the G1/S transition that is believed to be regulated by Cdk2/cyclin E complexes. Finally, mutations that inactivate the ultimate substrates of these kinases, the Rb family of tumor suppressor proteins, are also frequent in human cancers (see Figure 2).
likely to cause improper distribution of chromosomes among daughter cells, thus leading to aneuploidity, a characteristic of most human tumors.

Our laboratory has decided to systematically mutate the main cell cycle Cdk's to gain genetic evidence about their individual role in driving the cell cycle as well as their putative compensatory function. Early studies (Rane et al., Nat. Genet. 22, 44, 1999; Tsutsumi et al., Mol Cell Biol., 19, 7011, 1999) have illustrated that Cdk4 activity is dispensable for most cell types, presumably due to compensation by the highly related Cdk6. An exception are certain endocrine cell types, mainly pancreatic beta cells and pituitary lactotrophs (Moons et al., Endocrinology, 143, 3001, 2002). These defects are cell-autonomous since re-expression of Cdk4 in these specific cell types restores their proliferative properties (Martin et al., Oncogene, 22, 5261, 2003). In culture, primary Cdk4 (–/–) MEFs are resistant to transformation by Ras oncogenes and to immortalisation by loss of the INK4a locus (Zou et al., Genes & Dev., 16, 2923, 2002). To determine the extent of compensation by Cdk6 in these Cdk4 null cells, we have now generated mice that do not express this kinase. Mice lacking Cdk6 (–/–) mice are viable and do not display obvious abnormalities except for reduced populations of red blood cells.

Concomitant loss of both D-type cyclin-dependent Cdk4 and Cdk6 results in late embryonic lethality. Yet, these double mutant embryos develop rather normally until mid-gestation, do not show significant developmental abnormalities and display normal levels of cell proliferation and apoptosis. Lack of viability of these mice is most likely a consequence of their drastically reduced levels of red blood cells. A defect due to the limited proliferative properties of Cdk4/Cdk6 double null embryonic reticuloblasts. Surprisingly primary double mutant MEFs proliferate normally. These cells undergo culture crisis and become immortal upon continuous passage in culture. Moreover, Cdk4/Cdk6 double null MEFs exit from quiescence induced by serum removal with normal kinetics, albeit less efficiently (about 50%) than wild type MEFs. These observations challenge the presumed role of these kinases in facilitating cell cycle re-entry of mammalian cells.

Limited expression of these Cdk's has distinct consequences. Expression of a single Cdk6 allele in Cdk4(–/–);Cdk6(+/–) mice mainly exacerbates the defects observed in Cdk4 null mice. In contrast, expression of a single Cdk4 allele in
Cdk4(+/-);Cdk6(-/-) mice leads to the appearance of highly metastatic lymphoid leukemia in most animals. Thus, raising the possibility that Cdk6 may play a tumor suppressor role that can be compensated by full but not limited expression of Cdk4. Interestingly, Cdk6 null MEFs exit crisis earlier than wild type fibroblasts and can be more efficiently transformed by Ras and E1A oncogenes. Loss of Cdk4 expression in Cdk6 (-/-) MEFs does not affect this phenotype in spite of the fact that Cdk4 (-/-) MEF are resistant to Ras transformation. These observations suggest that the increased susceptibility to transformation induced by loss of Cdk6 has a dominant effect over the inhibitory effect caused by loss of Cdk4. Although the molecular mechanisms underlying these observations are still obscure, they clearly demonstrate that the physiological roles of the D-cyclin dependent Cdk4 and Cdk6 kinases is more complex than previously thought.

We have recently reported that in spite of current dogma, Cdk2 is essential for meiotic division but appears to be completely dispensable for mitotic cell division (Ortega et al., Nat. Genetics, 35, 25, 2003). These observations are consistent with related work involving mice lacking cyclin E1 and E2 (Geng et al., Cell, 114, 431; Parisi et al., EMBO J., 22, 4794, 2003). Yet, cyclin E deficient mice have a defect in trophoblast proliferation not observed in our Cdk2 null mice suggesting that cyclinE may associate with other, as yet unknown partners. These observations suggest that many of the key roles attributed to Cdk2 must be compensated by other kinases, possibly Cdk1. Cdk2 activity is regulated negatively by the Cip/Kip family of proteins. In my presentation, I will discuss emerging data that suggest that Cdk2 is not an essential partner for two members of this family, p21Cip1 and p27Kip1, two well-known tumor suppressors.

Finally, in collaboration with Dr. Javier Cáceres (Univ. of Glasgow), we have generated mice defective for the mitotic kinase Cdk1. Cdk1 null mice are not viable. Moreover, embryonic cells lacking Cdk1 do not proliferate to the morula stage, indicating an essential role for Cdk1 in the earliest stages of embryonic development and suggesting that perhaps Cdk1 is essential for cell proliferation. Further characterization of the mice described here as well as of their cells propagated in culture should shed light regarding the intimate roles that Cdk's play in driving and controlling the mammalian cell cycle.