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Review

New insights into cyclins, CDKs, and cell cycle control

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Abstract

Since their initial discovery in yeast, cyclin-dependent kinases have proven to be universal regulators of the cell cycle in all eukaryotes. In unicellular eukaryotes, cell cycle progression is principally governed by one catalytic subunit (cyclin-dependent kinase) that pairs with cell cycle-specific regulatory subunits known as cyclins. Progression through a specific phase of the cell cycle is under the control of a specific class of cyclin. Cell cycle control in multicellular eukaryotes has an additional layer of complexity, as multiple CDKs and cyclins are required. In this review, we will discuss recent advances in the area of cyclins and CDKs, with emphasis on the role of the mammalian proteins in cell cycle control at the cellular and at the organismal level. Many recent surprises have come to light recently as a result of genetic manipulation of cells and mice, and these findings suggest that our understanding of the intricacies of the cell cycle is still rudimentary at best.

Keywords: Cyclin; CDK; Cell cycle; Kinase; Substrate

Contents

1. Introduction

Early studies with cultured mammalian cells concluded that progression through the cell cycle was governed by several families of cyclin-dependent kinases, each pairing with a separate class of cyclin, most of which have at least two members [1,2]. One central theme emerging from these studies was that cell cycle progression required a different cyclin-dependent kinase for progression through each stage of the cell cycle. Thus, CDK1 together with cyclin B1 governs the G2/M transition. Exit from G1, in contrast, was found to be primarily under the control of cyclin D/CDK4/6. Finally, two other cyclins (A

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and E) that paired with CDK2 were found to be required for the G1/S transition and progression through S phase. These experiments—many of them using dominant–negative mutants or antibody microinjection—suggested functional, compartment-specific distinctions for cyclin and CDK family members and led many to predict that these genes would be essential for cell proliferation and overall organismal viability. In the first part of this review, we will summarize the major results from gene knockout studies focusing on each of the major cyclin/CDK complexes regulating cell cycle progression. These studies have revealed many surprises and have suggested that in many cases, other CDKs may compensate for the ablation of a particular cyclin/CDK complex in the context of the whole animal. In addition, this approach has revealed novel tissue-specific functions for cyclins and CDKs. Next, we will discuss our current knowledge of CDK substrates, with emphasis on those most recently discovered, and how they are regulated by this protein kinase family in vivo. Finally, we will discuss the most promising approaches currently available for the identification of protein kinase substrates. These studies have provided new insights into the age-old question of how cell cycle progression is coordinated in mammalian cells.

2. Gene knockouts: what do we really know?

2.1. G1-to-S transition, CDK4, CDK6, and cyclin D knockouts

Studies using cultured cells have indicated that passage through the G1/S transition is regulated by cyclin D/CDK4/6. One very well studied substrate of cyclin D1/CDK4 is the retinoblastoma tumor suppressor protein, pRB. The phosphorylation of pRB in G1 by cyclin D/CDK4/6 (and subsequently by CDK2) is believed to be a requisite event in reversing the repressive effects of pRB and de-repressing transcription of a number of genes required for exit from G1 and initiation and completion of S phase. It has been further suggested that phosphorylation of pRB by cyclin D/CDK4/6 initiates a subsequent round of phosphorylation of the tumor suppressor by CDK2 [3]. This has suggested that CDK4/6 and CDK2 do not have overlapping functions. These models have now been tested in the mouse by knocking out CDK4, CDK6, and the D-type cyclins singly or in combination. The results have revealed interesting, tissue-specific differences between CDK4 and CDK6 and surprisingly have challenged the notion of an absolute requirement for CDK4/6 in cell cycle progression.

CDK4 null mice are viable, much smaller than wild-type animals, and exhibit endocrine tissue-specific defects that result in diabetes and infertility [4,5]. Cell cycle progression defects at first were not readily apparent as CDK4$^{-/-}$ mouse embryonic fibroblasts (MEFs) grow and divide normally. S phase entry is, however, delayed by several hours upon cell cycle re-entry from quiescence [5]. CDK6$^{-/-}$ mice are also viable, but are of normal size. Deletion of this gene results in splenic and thymic hypoplasia [6]. In contrast, CDK6$^{-/-}$ MEFs proliferate normally. Resting CDK6$^{-/-}$ T lymphocytes, however, display a delayed response to mitogens. CDK6 may, therefore, have an important regulatory role in the proliferative response of T lymphocytes These studies have raised the question as to which CDK(s) substitutes for CDK4 or CDK6 in cells null for either gene or in the double knockout. For the CDK6 null, the most likely candidate would be CDK4, as mice lacking CDK6 and CDK2 are viable and exhibit no other defects in cell cycle progression other than those described for individual deletions of these genes [6]. In the case of CDK4, one obvious candidate would be CDK6, since it is the only other CDK known to interact with the D-type cyclins under physiological conditions. CDK6 could theoretically provide most of the CDK4 functions needed for cell cycle progression, such as pRB phosphorylation. However, the knockout mice for these genes clearly demonstrate that they do not have completely overlapping functions. CDK6 cannot, for example, compensate for the absence of CDK4 in the pancreas of CDK4 null mice. What then is the predominant pRB kinase (s) under these circumstances? Perhaps, in the absence of CDK4 and CDK6, some or all of the D cyclins could pair with CDK2 to produce a pRB kinase. In vitro, CDK2 bound to cyclin A or E can also function to phosphorylate pRB. Thus, it would be interesting to see whether a cyclin D/CDK2, cyclin A/CDK2 or cyclin E/CDK2 or a combination of one of these three kinases are utilized in CDK4 or CDK6 null cells for G1 exit and S phase entry. This issue has begun to be addressed by the recent generation of CDK4/6 null mice [6]. Mice null for these genes are viable but die shortly after birth. The embryos also display prominent defects in the hematopoietic system. These data indicate that CDK4 and CDK6 are not essential for embryogenesis. Biochemical studies of CDK4/6 null mouse embryo fibroblasts have further revealed that neither of these genes are required for entry or exit from quiescence [6]. However, the kinetics of cell cycle re-entry in these MEFs, while similar to that of wild-type MEFs, occurs in only a fraction of the population. Presently, the molecular basis for this heterogeneity in the population is not understood. Remarkably, pRB phosphorylation in CDK4/6 null MEFs during the G1 phase of the cell cycle, appears to have been assumed in part by CDK2 pairing with some of the D cyclins. Induction of cyclin D1 was observed in serum-stimulated CDK4/6 null MEFs, and cyclin D2/CDK2 complexes were shown to phosphorylate RB in vitro. The relative contributions of CDK2/cyclin A/E/D complexes to total RB phosphorylation were not, however, fully explored in this study. Thus, the extent to which CDK2/D cyclin complexes actually contribute to cell cycle progression in CDK4/6 null cells has not yet been resolved. What is clear is that under these conditions, CDK2 becomes essential for proliferation as shRNA-specific for CDK2 inhibits the proliferation of CDK4/6 null cells [6].

Disruption of the *cyclin D1* gene, like the loss of *CDK4*, leads to reduced size and is not lethal [7]. Thus, as in the