public health importance. While smokers may have the right to smoke, nonsmokers should have the right to be protected from harm resulting from the action of smokers. Reduction of exposure to ETS should be the goal of all nations.

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Cell cycle progression without cyclin E/CDK2: Breaking down the walls of dogma

G1 is the phase of the cell cycle wherein the cell is responsive to growth factor-dependent signals. As such, G1 regulation is frequently disrupted in cancer through deregulation of cyclin/CDK activity; deregulation of G1 phase provides tumorigenic cells with a growth advantage. Cyclin E, the regulatory cyclin for CDK2, is considered a requisite regulator of G1 progression. Cyclin E is overexpressed in cancer, suggesting that cyclin E/CDK2 deregulation contributes to tumorigenesis. Two papers now challenge both the concept that cyclin E/CDK2 is a requisite component of the cell cycle machine and efforts to develop cyclin E/CDK2 inhibitors as antiproliferative therapeutics.

The E type cyclins and their catalytic partner, CDK2, participate in the regulation of retinoblastoma protein inactivation, establishment of the prereplication complex (pre-RC), and initiation of S phase (Figure 1); their participation in these critical regulatory steps has resulted in the assumption that both cyclin E and CDK2 are indispensable for cell cycle progression. Support for this notion was initially provided by experiments utilizing a dominant negative CDK2 molecule to demonstrate that CDK2 activity is required for cell cycle progression of certain tumor-derived cell lines (van den Heuvel and Harlow, 1993). An essential role for E/CDK2 has two critical implications. First, as an essential enzyme, loss of either component should impede cell cycle progression. Second, as unchecked cell proliferation is a hallmark of human cancer, the cyclin E/CDK2 kinase should be a logical target for the development of anticancer therapeutics. In point of fact, cyclin E is overexpressed in human breast cancer, and its overexpression correlates with poor prognosis (Keyomarsi et al., 2002). However, two papers now challenge the notion that the cyclin E/CDK2 kinase is an essential component of the cell cycle machine.

In one approach, cyclin E was eliminated from the mouse via targeting of both genes encoding E type cyclins, cyclins E1 and E2 (Geng et al., 2003), and in the second, CDK2 itself was disrupted (Ortega et al., 2003). While the phenotypes are not entirely overlapping as one might expect, they do culminate with the startling revelation that neither E type cyclins nor CDK2 are strictly required for either embryonic development or for continuous cell cycle progression.

As with elimination of another G1 cyclin, cyclin D1 (Sicinski et al., 1995), the elimination of E type cyclins resulted in focal abnormalities. Defects were observed in the development of cell types that required repeated rounds of endoreplication (repeated rounds of S phase without intervening cell division) such as trophoblast giant cells. Such a phenotype might have been anticipated from earlier examination of cyclin E function in *Drosophila* development (Sauer and Lehner, 1995). Surprisingly, CDK2 ablation did not result in apparent defects in endoreplication cycles. Defects were also observed in spermatogenesis in $E2^{-/-}$ and $E1/E2^{-/-}$ mice that resulted in eventual male sterility. $CDK2^{-/-}$ mice, like cyclin E deficient mice, also exhibited defects in male spermatogenesis. Additionally, $CDK2^{-/-}$ mice also exhibited defective female gametogenesis, implicating the cyclin E/CDK2 kinase in the regulation of meiotic cell cycles. While it is far from settled,

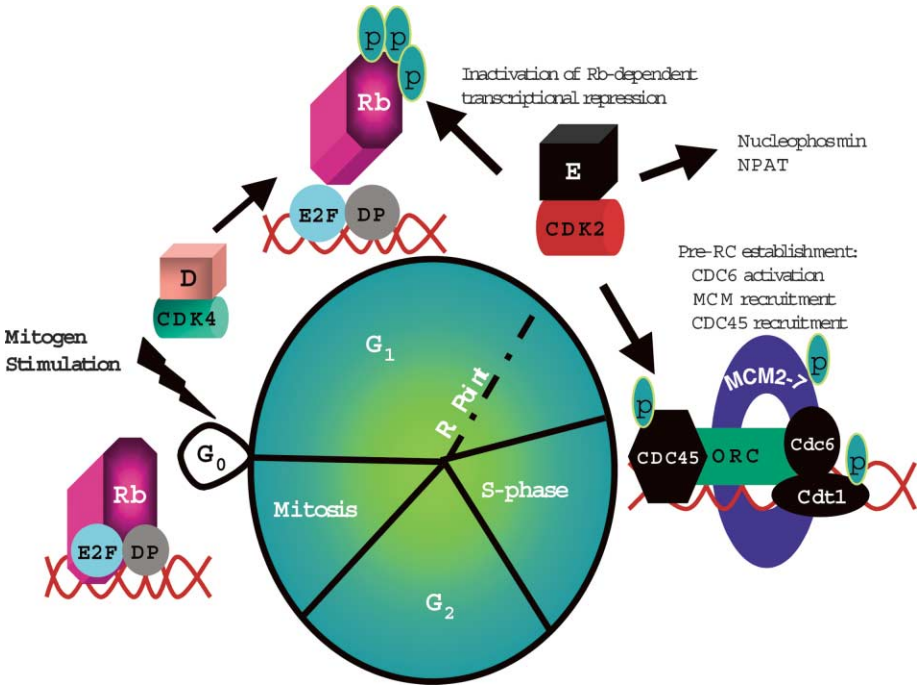

Figure 1. Regulatory activities of the cyclin E/CDK2 kinase

G1 to S phase progression is coordinated by the sequential activities of the cyclin D-dependent kinase and the cyclin E/CDK2 kinase. The D type and E type kinases function to sequentially inactivate the transcriptional repression activity of Rb family proteins prior to restriction point (R point) passage. The cyclin E/CDK2 kinase is then thought to perform a number of additional functions necessary for S phase entry. These include phosphorylation of nucleophosmin, which is necessary for centrosome duplication, phosphorylation of NPAT, which facilitates histone synthesis, and establishment of prereplication complexes (pre-RC) at origins of DNA replication. While cyclin E/CDK2 complexes have been demonstrated to phosphorylate a number of the proteins in the pre-RC, the functional importance of these phosphorylation events remains to be established.

the fact that CDK2 ablation fails to result in the defective endoreplication cycles observed in the E knockout suggests that E type cyclins have CDK2-independent functions (see below).

Is removal of the cyclin E/CDK2 kinase really of no consequence to cell cycle progression? To address this issue, both groups established primary cultures of embryonic fibroblasts (MEFs). Both cyclin E- and CDK2-deficient MEFs exhibited normal cell cycle distributions under conditions of continuous proliferation. The lack of any detectable phenotype under these conditions potentially results from functional redundancy among the various CDK complexes. While arguments could be made regarding whether a cyclin D/CDK4 or a cyclin A/CDC2 complex is the preeminent redundant kinase, it is likely that both contribute in the absence of a functional cyclin E/CDK2 kinase.

While no defect was noted in continuously proliferating cells, when cell cycle reentry was examined, defects were observed, and the cohesion between the E1/E2$^{-/-}$ and CDK2$^{-/-}$ phenotypes once again blurs. While G0 to S phase progression appears essentially unperturbed in CDK2$^{-/-}$ fibroblasts, E1/E2$^{-/-}$ MEFs fail to reenter the cell cycle. Mechanistically, the defect in the cyclin E-deficient cells results from the inability of these cells to fully establish a pre-RC at origins of replication. Formation of the pre-RC requires the ordered assembly of multiple proteins at sites of DNA replication initiation (Bell, 2002). The origin recognition complex binds first, followed by CDC6 and cdt1, which together recruit the hexameric minichromosome maintenance complex (MCM2-7), the putative replicative helicase. The development of cell-free replication assays has revealed that the cyclin E/CDK2 kinase functions synergistically with CDC6 to facilitate MCM loading (Coverley et al., 2002). The inability of the MCM hexamer to associate with chromatin during cell cycle reentry supports the notion that cyclin E performs an essential, nonredundant role in pre-RC establishment.

While the defect in MCM loading in the E1/E2$^{-/-}$ MEFs confirms previous work, the fact that no such defect is observed in CDK2$^{-/-}$ cells is paradoxical. Several potential mechanisms can be envisioned that could resolve this issue. The first is that another G1 CDK functions in its absence. Consistent with this idea, the cyclin D1/CDK4 kinase does associate with the pre-RC (Gladden and Diehl, 2003). However, it remains to be demonstrated that a cyclin D1/CDK4 complex can actually drive mature pre-RC formation. A second possibility is that cyclin E binds to another CDK, such as CDK3, to perform its critical function. However, the CDK3 gene is disrupted by a premature termination codon in most strains of laboratory mice, eliminating it as a potential partner (Ye et al., 2001). A third possibility is that cyclin E can associate with an as yet unidentified kinase and thereby promote S phase. However, lack of detectable catalytic activity associated with cyclin E in the CDK2$^{-/-}$ cells argues against this possibility. Finally, it is possible that E type cyclins have an intrinsic CDK-independent function that is wholly unrelated to their capacity to promote activation of an established protein kinase.

There are currently a number of small molecular weight compounds that target CDK2. If proliferating cells can circumvent CDK2 loss or inhibition, will therapeutics that target the CDK2 kinase be efficacious? The capacity of cells lacking either cyclin E or CDK2 to continuously proliferate suggests that normal somatic cells should withstand therapeutics that specifically target CDK2 with little adverse effect. The question is, will tumor cells display increased sensitivity to CDK2 inhibition, perhaps due to their inherently increased proliferative capacity? The continued proliferation of colon cancer derived cell lines following inhibition of CDK2 (Tetsu and McCormick, 2003) argues that, at the very least, certain cancer types could be quite refractory to CDK2-based therapies. The resistance of the colon cancer cells to CDK2 inhibition likely reflects redundancy between G1 cyclin-dependent kinases; given that cyclin D1 is frequently overexpressed in colon cancer, its deregulation could drive cell cycle progression in the absence of the cyclin E/CDK2 kinase. Still, if CDK2 inhibitors

are not effective single agent therapeutics, perhaps they will function in combination with standard chemotherapeutic approaches. Inhibition of the cyclin E/CDK2 kinase could potentially sensitize tumor cells and increase the tumor-specific killing potential of more traditional treatment regimes.

A final intriguing phenotype observed in both the E and CDK2 ablated mice was the resistance of MEFs derived from these mice to oncogene-mediated transformation. Their resistance points toward the potential use of CDK2-based therapies as chemopreventive agents. While this approach might not be generally applicable to all individuals, it might provide some benefit to those with a known genetic predisposition to cancer. Specific inhibition of CDK2 in these individuals might help prevent the overt transformation of cells sustaining a "second hit."

The work of Geng et al. and Ortega et al. challenges the current concepts regarding the molecular basis of cell cycle progression and suggests a reexamination of the utility of targeting a specific CDK for cancer therapy. Not only does this new data challenge many established paradigms, but it also introduces a new challenge. Can one distinct molecule be targeted to block cell cycle progression in the tumor cell? So far, the answer is no, most likely reflecting the high degree of built-in redundancy. Efficacious therapies will have to address these redundancies, possibly through the targeting of more than a single step in a given pathway. As our knowledge of the molecular processes that drive cell proliferation evolves, so should our ability to design antiproliferative agents.

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