The cell cycle of mammalian cells is divided into four phases: G₁ (first gap), S (DNA synthesis), G₂ (second gap), and M (mitosis). Quiescent cells that have not entered the cell cycle are referred to as being in G₀. Progression through the cell cycle requires the activation of cyclin-dependent kinases (CDKs). CDK activation is dependent on the association of the CDK with a cyclin regulatory subunit. Cyclin D/CDK4, cyclin D/CDK6, and cyclin E/CDK2 regulate transition through G₁, cyclin A/CDK2 regulates S phase transition, and cyclin A/CDK2 and cyclin B/CDK2 regulate G₂/M transition, as shown by the Figure. The activity of CDKs is also regulated by endogenous CDK inhibitors (CKIs) in the cyclin/CDK complex (Figure). Two families of CKIs have been characterized according to their structures and CDK targets. Kip/Cip proteins (p21Cip1, p27Kip1, and p57Kip2), which bind to both cyclin and CDK subunits, inhibit cyclin E- and A-dependent kinases but act as positive regulators of cyclin D-dependent kinases. The INK family of proteins (p16INK4a, p15INK4b, p18INK4c, and p19INK4d) exclusively binds to and inhibits CDK4 and CDK6.¹

Initiation of the cell cycle occurs when a quiescent cell (in G₀) is stimulated with appropriate mitogenic stimulus. This event induces expression of D-type cyclins that bind to CDK4 and CDK6, and then enter the nucleus. Active cyclin D-dependent kinases phosphorylate the retinoblastoma protein (Rb) in mid-G₁.¹ Rb is then phosphorylated on additional sites by the cyclin E/CDK2 complex, and this event leads to disruption of Rb association with the transcription factor complex E2F, release of active E2F, and subsequent transcription of genes necessary for DNA synthesis, such as DNA polymerase α and thymidine kinase.² Cyclin A- and B-dependent kinases maintain Rb in a hyperphosphorylated form during S, G₂, and M.¹

**Cell Cycle Progression and Cell Migration Are Closely Linked**

Arterial smooth muscle cells (SMCs) in the normal arterial wall are quiescent and stationary. It is believed that injury of the artery, for example, by mechanical means, inflammatory processes, hypertension, or diabetes, causes various cytokines and growth factors to "activate" the SMCs, thereby increasing growth factor receptor expression and the ability of the SMC to proliferate and migrate.³ Thus, proliferation and migration of SMCs are commonly believed to contribute to atherosclerosis, and restenosis after coronary angioplasty and stenting.⁴,⁵ Proliferation and migration of SMCs also occur during normal development of the cardiovascular system. It has long been known that quiescent SMCs have a low ability to undergo migration and chemotaxis,⁶ and that many growth factors induce both cell proliferation and cell migration. A central question in vascular biology is whether the cell cycle regulates the ability of cells to move.⁷ Several observations show that there is a close link between cell cycle progression and cell migration. For example, it has been demonstrated that rapamycin, a macrolide antibiotic, inhibits both G₀ to S transition and migration of SMCs,⁸ and that this effect is reduced in SMCs derived from p27Kip1-deficient mice,⁹ suggesting a role for CDKs and p27Kip1 in SMC migration. However, rapamycin also induces effects that are independent of p27Kip1.¹⁰ Overexpression of any one of p27Kip1, p21Cip1, or a nonphosphorylatable form of Rb results in reduced neointimal thickening after angioplasty in rodents and pigs.¹¹⁻¹⁵ This effect is due, at least in part, to reduced SMC proliferation. Although SMC migration is difficult to measure in in vivo models, due to lack of specific markers, the reduced neointimal thickening observed after overexpression of p27Kip1, p21Cip1, or the Rb mutant most likely also is due to reduced SMC migration from the media into the neointima.

In this issue of *Circulation Research*, Díez-Juan and Andrés¹⁶ extend these observations by showing that migration of rat SMCs and NIH-3T3 cells is directly dependent on CDK activity and downstream Rb phosphorylation. In keeping with previous studies,⁹,¹⁷ overexpression of physiologically relevant levels of p27Kip1 led to reduced cell migration. The following new observations are now provided by Díez-Juan and Andrés.¹⁶ First, overexpression of a mutant of p27Kip1 with impaired CDK inhibitory activity did not inhibit cell migration or proliferation, which indicates that the inhibitory effects of p27Kip1 are likely to be mediated by reduced CDK activity. Second, forced expression of a phosphorylation-deficient Rb mutant insensitive to CDK-mediated inactivation and subsequent E2F activation (see Figure) led to reduced cell proliferation and cell migration. Third, overexpression of the E1A oncprotein, which associates with Rb and results in release of E2F from Rb in a CDK-independent manner, could overcome the inhibitory effects of overexpressed p27Kip1 on cell proliferation and cell migration without restoring CDK activity. These observations convincingly demonstrate that cell cycle events regulate cell migration.

How Do Processes Involved in the Cell Cycle Lead to the Generation of Cell Movement?

It appears that the maximal potential for a given cell, including a SMC, to migrate coincides with the mid-late G₁,
phase, whereas cells in late S or G2/M have a lower, or no, ability to move.18–22 Accordingly, many agents and gene products that block SMC proliferation also inhibit migration of SMCs.8,23–27 How is this coordinated regulation of cell proliferation and cell migration mediated? One likely possibility, suggested by the studies of Díez-Juan and Andrés, is that specific signals required for G1/S transition, such as CDK2 activation and release of E2F after Rb phosphorylation, directly affect events required for cell migration. Indeed, cell cycle arrest induced by overexpression of p27kip1 correlated with the loss of lamellipodia formation, actin reorganization, and focal adhesion reorganization, without affecting cell adhesion.16 Interestingly, there appears to be a close link between cell cycle progression, cell migration, and expression of the integrin vitronectin receptor α5β1.23,28 This could provide one mechanism for the regulation of cell movement by the cell cycle. Still, it is not known if there is a direct effect of E2F on signaling events required for cell migration, or if the effects of agents that coordinateably regulate cell proliferation and cell migration are due to an indirect, “inherent,” ability of cells in late G1 to migrate. This question is difficult to address because overexpression of cell cycle regulatory molecules affects the proportion of cells in G1, S, G2, and M. Specific E2F-regulated targets required for cell migration will have to be identified. Regardless of the molecular pathways that link G1 progression and cell migration, there is now strong evidence that these two processes are closely coordinated. These findings have important clinical implications, for example, in relation to the promising use of coronary stents coated with growth-suppressing agents, such as rapamycin (sirolimus).29 Growth-suppressing agents are now predicted to also inhibit SMC migration in vivo by causing cell cycle arrest.

References


**Key Words:** migration ■ cyclin-dependent kinase inhibitor ■ retinoblastoma protein ■ smooth muscle cells
The Cyclin-Dependent Kinase Pathway Moves Forward
Karin E. Bornfeldt

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