Integrins and cell proliferation: regulation of cyclin-dependent kinases via cytoplasmic signaling pathways

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Summary
Cell cycle progression in mammalian cells is strictly regulated by both integrin-mediated adhesion to the extracellular matrix and by binding of growth factors to their receptors. This regulation is mediated by G1 phase cyclin-dependent kinases (CDKs), which are downstream of signaling pathways under the integrated control of both integrins and growth factor receptors. Recent advances demonstrate a surprisingly diverse array of integrin-dependent signals that are channeled into the regulation of the G1 phase CDKs. Regulation of cyclin D1 by the ERK pathway may provide a paradigm for understanding how cell adhesion can determine cell cycle progression.

Key words: Tyrosine kinase, Signal transduction, Growth control, Cell adhesion

Introduction
Proliferation of mammalian cells is tightly regulated by multiple environmental influences, primarily adhesion to the extracellular matrix (ECM), cell-cell adhesion and soluble factors (i.e. polypeptide growth factors or inhibitors, mitogenic lipids, inflammatory cytokines and hormones). Of these environmental cues, soluble growth factors and integrin-mediated adhesion are most crucial, loss of adhesion generally resulting in complete G1 phase cell cycle arrest (Stoker et al., 1968). For susceptible cell types, loss of adhesion leads to apoptosis (reviewed in Giancotti and Ruoslahti, 1999), which might be regarded as the extreme case in which cell numbers decrease rather than remain stable. Conversely, formation and spread of tumors is closely associated with decreased dependence on ECM for growth and survival. Thus, loss of tumor suppressors or constitutive activation of proto-oncogenes not only leads to elevated growth rates but also enables tumor cells to colonize inappropriate environments (reviewed by Schwartz, 1997). Not only are many different integrins required for survival of multicellular organisms, but genetic deletion of several of the major intracellular proteins that transduce integrin signals also results in early embryonic lethality (Ilic et al., 1995; Honda et al., 1998). Both integrins and growth factor receptors use multiple cytoplasmic signaling pathways to regulate G1 phase cyclins and associated kinases that determine cell cycle progression. The many cases where integrins potentiate signaling by other receptors have contributed to the growing recognition that signaling pathways are highly interwoven into complex networks. Here, we focus on recent advances in our understanding of how integrins, in cooperation with tyrosine kinase growth factor receptors (RTKs), regulate the signal transduction pathways that ultimately control the G1 phase cyclin-dependent kinases (CDKs).

Integrin-dependent signaling pathways
Signal transduction by integrins is initiated by both occupancy and crosslinking of integrins by ECM proteins (Schwartz et al., 1991; Kornberg et al., 1991; Miyamoto et al., 1995; Shankar et al., 1993). These events lead to changes in activity of most if not all of the known growth regulatory pathways (reviewed by Giancotti and Ruoslahti, 1999; Cary et al., 1999). The list is long but includes protein tyrosine and serine/threonine kinase cascades, small GTPases, inositol lipid pathways and other phospholipid cascades. One potentially important aspect of integrin signaling stems from the fact that stimulation occurs at defined contacts, in contrast to stimulation by soluble factors, which can occur over the entire cell. Because integrins also cooperate with growth factor receptors to regulate downstream events, localized adhesions have the potential to impose a measure of spatial specificity on otherwise global growth-factor-induced events. This level of regulation is likely to be important for cell migration or tissue organization, but to what extent it contributes to cell cycle progression remains to be determined.

In the context of growth regulation, it is essential to distinguish transient effects seen in replated cells from sustained effects in stably adherent cells. Investigators have often studied integrin signaling by plating suspended cells on ECM proteins, which is analogous to acute stimulation of starved cells with growth factors. This protocol initiates transient stimulation of many pathways, including Rac, Cdc42 and their downstream kinases; tyrosine kinases or substrates such as Src and Shc; Ras and JNK; and phosphoinoside 3-kinase (PI3K) and its downstream pathways (for reviews see Giancotti and Ruoslahti, 1999; Cary et al., 1999). These events are likely to be important in regulation of cell migration, cytoskeletal organization or gene expression; however,
transient stimulation of pathways in early G_1 is generally not sufficient to induce S-phase entry. For example, ERK and PI3K must be active in mid-G_1 phase for cells to enter S phase, and transient PI3K activity in early G_1 phase is even dispensable for cell cycle progression (Meloche et al., 1992; Jones et al., 1999; Gille and Downward, 1999; Jones and Kazlauskas, 2001). Similarly, Bohmer et al. reported that cell adhesion is required throughout most of G_1 phase (Bohmer et al., 1996). Among the integrin-dependent signals characterized to date, stimulation of tyrosine phosphorylation of focal adhesion kinase (FAK), p130^{cas} and paxillin, PIP 5-kinase activity and Na^{+}/H^{+} antiporter activity are sustained as long as cells remain adherent (reviewed by Schwartz et al., 1995). There are older data suggesting that Na^{+}/H^{+} exchange is important (Schwartz et al., 1995), but in most cases causal relationships between these sustained integrin-dependent signals and the cell cycle machinery have yet to be established. FAK has been the most studied, and the results of studies linking FAK to G_1 phase cell cycle progression are outlined below.

Potentiation of growth factor receptor signaling by integrins also contributes critically to cell cycle progression. This cooperation begins at the level of the receptors themselves. Cell adhesion increases the number of PDGF receptors by blocking their degradation by a ubiquitin-dependent pathway (Baron and Schwartz, 2001). Integrins also physically associate with growth factor receptors and enhance receptor activation (Schneller et al., 1997; Jones et al., 1997; Moro et al., 1998).

Under many conditions, growth factor activation is substantial in non-adherent cells (McNamee et al., 1993; DeMali et al., 1999), in which cases integrins enhance downstream signaling. The ERK MAP kinase cascade is regulated by integrins at multiple points. One study showed that, although overall PDGF activation was unaffected by adhesion, phosphorylation at a single site and binding of RasGAP to the PDGFR was decreased in cells plated on FN compared to polylysine, leading to increased Ras and ERK activation on FN (DeMali et al., 1999). In other studies, activation of Ras was unaffected by adhesion, but activation of Raf and subsequently ERK was strongly adhesion-dependent (Lin et al., 1997; Howe and Juliano, 2000). Additionally, others reported that activation of MEK by Raf requires integrin-mediated adhesion (Renshaw et al., 1997), or that sustained activation of ERK by growth factors requires cell adhesion (Roovers et al., 1999). These discrepancies suggest that the ERK cascade has multiple adhesion-dependent steps whose importance varies between cells or experimental conditions. Nevertheless, it is agreed that sustained ERK activation requires cooperative signaling between RTKs and integrins.

Interestingly, FAK participates in multiple growth regulatory events. Integrin-mediated effects on FAK control transcription of the adapter protein IRS-1 (Lebrun et al., 2000). This effect appears to be mediated in part by effects on JNK activity, in general agreement with a previous study reporting that JNK mediates FAK regulation of cell cycle progression (Oktay et al., 1999). Although proliferation was not assayed, FAK also mediates integrin enhancement of signaling by growth factors to activate MEK and ERK (Renshaw et al., 1999). A recent paper showed that FAK can mediate activation of the ERK cascade in cells replated on fibronectin (FN) via Rap1 and B-Raf rather than Ras and Raf-1 (Berberis et al., 2000). However, this paper did not directly evaluate the integrin–growth-factor synergy pathway by comparing adherent and suspended cells. Finally, inhibition of protein kinase A delayed the decline in both FAK phosphorylation and Raf activity after cell detachment (Howe and Juliano, 2000). These results suggest that FAK is a multifunctional protein that plays a critical role in the integrin enhancement of mitogenic signaling.

A number of other RTK pathways are sensitive to cell adhesion. Growth factor induction of the Rac pathway is integrin dependent (del Pozo et al., 2000). In this context, RTKs still activate Rac in suspended cells, but the activated Rac fails to target to the plasma membrane and interact with its effectors. Activation of PI3K and Akt in response to EGF is also attenuated in non-adherent cells (Khwaja et al., 1997), as is growth factor activation of JNK (Short et al., 1998). Finally, PDGF activation of protein kinase C and downstream events was decreased in non-adherent cells, despite equivalent phosphorylation of phospholipase C (McNamee et al., 1993). In this system, detachment from the ECM led to a decline in activity of PIP 5-kinase and cellular levels of phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)^{2}), which is the substrate for phospholipase C. As PtdIns(4,5)^{2} is a major determinant of the actin cytoskeleton and membrane targeting of pleckstrin homology domain proteins (Toker, 1998), a decrease in its levels in suspended cells could have widespread effects on signaling pathways. Interestingly, actin polymerization itself is a determinant of gene expression through the serum-response element, which is found in the promoters of many cytoskeletal and cell cycle regulatory genes (Sotiropoulos et al., 1999). Integrins induce polymerization and organization of actin through both physical protein-protein interactions that anchor actin filaments at sites of adhesion and through signaling pathways such as PtdIns(4,5)^{2} and Rac family GTPases (reviewed by Schoenwaelder and Burridge, 1999). Thus, actin itself could also contribute to integrin effects on cell cycle progression. These findings are summarized in Fig. 1.

Finally, we note that, although the major model for integrin signaling is positive regulation due to ligation and crosslinking of integrins, several labs have observed that unoccupied integrin α5β1 in nonadherent cells has dominant effects that inhibit growth or survival (Giancotti and Ruoslahti, 1990; Symington, 1992; Varner et al., 1995). In an interesting twist, the CDK inhibitor INK4a was found to sensitize cells to detachment-induced apoptosis by increased transcription of integrin α5β1; the unoccupied integrin then promotes cell death (Plath et al., 2000). Thus, an alternative model is that unoccupied or unclustered integrins transmit signals that are terminated by integrin occupancy or clustering. Accordingly, the integrin β3 cytoplasmic domain was recently shown to interact with and activate caspase 8 to induce death of endothelial cells, and clustering negatively regulated this interaction (Stupack and Cheresh, submitted). Thus, although many studies show integrin stimulation of cytoplasmic pathways, these do not in principle distinguish direct activation from reversal of an inhibition. Both mechanisms probably contribute to cell survival and growth to some extent.

**Regulation of cyclins and CDKs**

Regulation of G_1 phase by CDKs is now fairly well understood (reviewed by Weinberg, 1995; Sherr et al., 1999). Of the major
components in the G₁ phase CDK system (cyclins D and E, CDK4, CDK2 and their inhibitors), integrin/RTK signaling has been most closely associated with the induction of cyclin D1 and the downregulation of the CDK inhibitors, p21cip1 and p27kip1 (reviewed by Roovers and Assoian, 2000). Cyclin D1 binds to CDK4 or CDK6, which leads to their activation. p21cip1 and p27kip1 bind to cyclin-E–CDK2 and cyclin-A–CDK2 complexes, leading to their inhibition. Activated CDK4/6 and CDK2 are required for phosphorylation of the retinoblastoma protein, an event that allows the induction of E2F-dependent genes and signifies the end of cells’ dependence on extracellular stimuli. Thus, regulation of cyclin D1, p21cip1 and p27kip1 by growth factors and the ECM ultimately commits the cell to division.

The induction of cyclin D1 mRNA has most frequently been attributed to the activation of ERKs (reviewed by Roovers and Assoian, 2000). In most cases, activation of the Ras-Raf-MEK-ERK cascade induces cyclin D1 gene expression. Conversely, inhibition of the Ras pathway inhibits cyclin D1 gene expression. Several studies have shown that the induction of cyclin D1 requires only moderate ERK activity, but the activity must be sustained for several hours (Weber et al., 1997). Sustained ERK signaling in response to integrin and RTK synergism (Roovers and Assoian, 2000) may therefore explain the joint growth factor plus ECM requirement for cyclin D1 expression.

A sustained ERK signal, although necessary, is not sufficient to induce cyclin D1 protein. Sustained ERK signaling increases cyclin D1 expression in adherent but not suspended CCL39 cells (Le Gall et al., 1998). Thus, adhesion must make other contributions to G₁ progression. One such contribution is evident from studies indicating that the transport of ERK from the cytoplasm to the nucleus is dependent upon cell adhesion (Danilkovitch et al., 2000). Alternatively, PI3K is required for the expression and stability of cyclin D1 (Gille and Downward, 1999; Takuwa et al., 1999; Diehl et al., 1998). As discussed above, both integrins and RTKs contribute to PI3K activity. The relative importance of ERK signal duration, ERK nuclear translocation and PI3K activity in the induction of cyclin D1 among different cell types remains to be determined.

Integrin signaling also stimulates the translation of cyclin D1 mRNA, and this effect seems to be the major mechanism by which integrins increase cyclin D1 levels in endothelial cells (Huang et al., 1998). Very recent studies indicate that activation of Rac by integrins is important for the translation of cyclin D1 in endothelial cells (F. Giancotti, personal communication), again suggesting that regulation of cyclin D1 expression is likely to involve more than MEK/ERK activation. Interestingly, integrins mediate the translocation of activated Rac to the plasma membrane (see above) and the movement of mRNA and ribosomes to focal adhesions (Chicurel et al., 1998b). These complementary effects suggest that subcellular compartmentalization by the ECM might integrate different integrin-dependent events affecting cell proliferation. Overexpression of activated Rac also stimulates cyclin D1 gene expression, at least in fibroblasts (Gille and Downward, 1999; Joyce et al., 1999; Page et al., 1999). Rac may therefore have cell-specific effects on the synthesis of cyclin D1 mRNA and protein. Rac activity and function are regulated by integrins (see above), which again suggests that regulation of cyclin D1 expression is likely to involve more than MEK/ERK activation.

A few studies have assessed the upstream signaling mechanisms by which integrins might regulate cyclin D1. Overexpression of wild-type and dominant negative FAK cDNAs showed that integrin-dependent phosphorylation of FAK plays an important role in the phosphorylation of ERK.
and induction of cyclin D1 (Zhao et al., 1998). The results with FAK agree well with studies implicating FAK in integrin/RTK-dependent ERK activation (Renshaw et al., 1999). Others have shown that overexpression of ILK leads to the expression of cyclin D1 (Radeva et al., 1997). Given that ILK is downstream of PI3K (Delcommenne et al., 1998), these results agree with studies implicating PI3K signaling in control of cyclin D1 expression.

In addition to the stimulation of cyclin D1 synthesis, integrin signals are important for the downregulation of CDK inhibitors of the p21 family, p21cip1 is induced in early G1 phase. Its induction requires strong ERK activity (reviewed by Roovers and Assoian, 2000), which is dependent upon synergistic signaling by growth factor receptors and integrins (discussed above). In mid-late G1 phase, the levels of p21cip1 and p27kip1 are downregulated coincidently with activation of cyclin-E–CDK2. This downregulation is impaired when integrin signaling is blocked (reviewed by Roovers and Assoian, 2000).

The mechanisms underlying these effects are poorly understood, but ERK seems not to be directly involved (Bottazzi et al., 1999; Olson et al., 1998). Several studies have implicated Rho in the downregulation of both p21cip1 and p27kip1 (Weber et al., 1997; Olson et al., 1998; Adnane et al., 1998; Laufs et al., 1999), but there is little evidence to support a direct link between integrin signaling and Rho activation. Indeed, direct analysis of Rho–GTP levels shows that integrins have complex effects on Rho activity and do not simply stimulate Rho activity throughout G1 phase (Ren et al., 1999).

**Integrin- and matrix-specific effects**

There are many indications that not all ECM-integrin interactions support cell cycle progression equally well. In some instances differences appear to be due to quantitative differences in integrin expression, avidity or affinity. Expression of integrin αvβ3, which binds vitronectin, osteopontin, fibronectin and other matrix proteins, is associated with growth or tumorigenesis of several cell types, as is deposition of vitronectin or osteopontin matrices that bind αvβ3 (reviewed by Varner and Cheresh, 1996). Integrin αvβ3 has been found to specifically associate with tyrosine kinase growth factor receptors and the downstream adapter IRS-1 (Schneller et al., 1997; Jones et al., 1997; Vuori and Ruoslahti, 1994; Soldi et al., 1999), and these effects correlate with enhanced proliferation on αvβ3 ligands compared with collagen. Interaction sites on the integrin appear to reside in the extracellular domain of integrin β3 for the PDGFR and VEGFR2, although the latter also required integrin αv (Borges et al., 2000).

Integrin αv is among those α subunits that, along with α5 and α1, associate with Shc and caveolin and promote DNA synthesis (Wary et al., 1996; Wary et al., 1998). These associations unexpectedly depend on sequences in the α subunit transmembrane and extracellular domains. Although the transient activation of ERK that occurs downstream of Shc appears to be too brief to account for transit through G1 phase, the association could mediate other growth-promoting signals. Integrin αvβ3 also shows a particular ability to cooperate with bFGF to promote long term ERK activation and angiogenesis (Elcieri et al., 1998), to activate the NF-κB pathway (Scatena et al., 1998) and to induce calcium entry into cells (Schwartz and Denningerhoff, 1994). In several of these studies, other integrins failed to induce similar effects despite similar abilities to promote adhesion and cytoskeletal organization.

In addition to integrin αvβ3, integrin α5β1 (the classical fibronectin receptor), associates with caveolin and Shc (as mentioned above) and also promotes DNA synthesis (Wary et al., 1996). In myoblasts, elevated α5β1 expression promotes ERK activation and proliferation, whereas elevated levels of integrin α6β1 (a laminin receptor) inhibit ERK and promote withdrawal from the cell cycle and differentiation (Sastry et al., 1999). In astrocytoma cells, adhesion to vitronectin or fibronectin promotes activation of PI3K and its association with FAK much better than does adhesion to collagen (Ling et al., 1999). Integrins α5β1 and αβ3 also promote responsiveness of vascular smooth muscle cells to insulin-like growth factor by a distinct mechanism: secretion of IGF binding protein-5 (IGFBP-5; Zheng et al., 1998). Adhesion of these cells to collagen or laminin promotes cell growth much less well but the difference is eliminated by addition of soluble IGFBP-5.

Different collagen receptors, however, have distinct growth regulatory and signaling properties. Integrin α1β1 in skin fibroblasts promotes cell proliferation, apparently by the caveolin-dependent signaling pathway discussed above (Pozzo et al., 1998). By contrast, stimulation of integrin α2β1 does not promote proliferation in endothelial cells and fibroblasts (Wary et al., 1996; Pozzo et al., 1998; Davey et al., 1999). Interestingly, integrin α2β1 activates p38 MAP kinase (Ivaska et al., 1999), and p38 is thought to inhibit transcription of the cyclin D1 promoter (Lavoie et al., 1996). However, the inhibitory effect of integrin α2β1 on cell proliferation may be cell-type specific; α2β1 promotes mammary epithelial tube formation and proliferation more effectively than does integrin α1β1 (Zutter et al., 1999). These findings are summarized in Fig. 2.

**Organization of the extracellular matrix**

In vivo, cells interact with ECM proteins in polymeric matrices whose macromolecular organization and mechanical properties can be important determinants of cell cycle progression. Plating cells on a mutant FN, FNIII1-7, that contains all of the known cell-binding motifs but forms a structurally distinct matrix induces less efficient phosphorylation of FAK and progression through G1 phase compared with wild-type FN (Sechler and Schwartzbauer, 1998). When cells were treated with a 76-residue fragment of FN (III1-C) that forms one of the self-assembly sites and inhibits FN matrix assembly, the FN matrix was disassembled and the activation of cyclin-E–cdk2 was blocked (Bourdoulous et al., 1998). Cell adhesion to FN that contains the alternatively spliced EDA segment produced more efficient progression through G1 phase (assessed by phosphorylation of ERK, induction of cyclin D1, phosphorylation of pRb and entry into S phase) than did FN lacking EDA (Manabe et al., 1999). However, at least some of these effects may be secondary to increased rates of cell adhesion and spreading. Indeed, Huang et al. reported that cell spreading on FN, rather than cell adhesion per se, is the critical determinant underlying the adhesion dependency of cyclin D1 mRNA translation and the downregulation of p27kip1 in human capillary endothelial cells (Huang and Ingber, 1999).
A few studies have examined the role of the collagen matrix in cell proliferation. Aortic smooth muscle cells proliferate poorly on polymerized collagen gels (conditions in which cell spreading is minimal), which correlated with poor downregulation of p27<sup>kip1</sup> (Koyama et al., 1996). Conversely, monomeric collagen films supported efficient cell spreading, p27<sup>kip1</sup> downregulation and G<sub>1</sub> phase cell cycle progression. Subsequent studies in this system revealed that FN/α5β1 binding mediates the proliferation of smooth muscle cells on collagen films and that the inefficient proliferation of smooth muscle cells on collagen gels reflects impaired formation of FN fibrils and downregulation of integrin α5β1 (Raines et al., 2000; E. W. Raines et al., personal communication). These new results fit nicely with studies indicating that cell spreading on collagen, in the absence of FN/α5β1 signaling, does not support cell proliferation (Wary et al., 1996; Davey et al., 1999).

In addition to chemical signaling, integrin ligation results in the organization of the actin cytoskeleton and generation of tensile forces (mechanical signaling) that contribute to G<sub>1</sub> phase progression (reviewed by Huang and Ingber, 1999; Koyama et al., 1996; Raines et al., 2000; Chicurel et al., 1998a). Early studies showed that when hepatocytes are plated on increasing amounts of fibronectin, attachment per se is sufficient to promote progression through early G<sub>1</sub> phase but cell spreading is required to complete progression into S phase (Hansen et al., 1994). Subsequently, micropatterned matrices were used to demonstrate that the effect of cell spreading is independent of the density of integrin clusters in capillary endothelial cells (Chen et al., 1997). More recently, these techniques were used to show that cell spreading is required for the translation of cyclin D1 and downregulation of p27<sup>kip1</sup> in endothelial cells (Huang et al., 1998). These results support the premise that the proliferative effect of cell spreading reflects the development of mechanical tension within the cell that is specifically dependent on the ligation of integrins (Huang et al., 1998; Chicurel et al., 1998).

The Grinnell laboratory has studied effects of mechanical tension in fibroblasts within collagen gels. When cells are embedded within gels that resist tensional forces, ERK is phosphorylated, cyclin D1 is expressed, p27 is downregulated and the cells cycle when stimulated with growth factors. Disruption of mechanical tension leads to the loss of actin stress fibers, the inactivation of ERKs, the loss of cyclin D1 and the upregulation of p27<sup>kip1</sup> (Rosenfeldt and Grinnell, 2000; H. Rosenfeldt and F. Grinnell, personal communication). Several laboratories have used cytochalasin D to disrupt the actin cytoskeleton and cell spreading, and these experiments typically result in an inhibition of integrin signaling and integrin-dependent cell cycle progression. However, when fibroblasts are cultured in collagen gels, cell spreading can persist even when stress fibers have been disassembled. Using this approach, the Grinnell lab showed that cell spreading in the absence of actin stress fibers is not sufficient to support ERK activation, cyclin D1 expression, p27<sup>kip1</sup> downregulation or cell proliferation. Others studies discussed above also indicate that cell spreading itself is not sufficient to support proliferation (Wary et al., 1996; Davey et al., 1999). Together, the results to date support the idea that both chemical and mechanical signals play roles in stimulating progression through G<sub>1</sub> phase.

Mechanical and chemical signaling appear to be linked. Cells respond to mechanical forces by activating a substantial range of signaling pathways, including the ERK and JNK pathways (reviewed by Shyy and Chien, 1997). Among these effects, increasing mechanical tension stimulates FAK phosphorylation (Yano et al., 1996; Tang et al., 1999). Cell-generated tension might therefore feed back on focal adhesions to influence the activity of FAK, which could promote cell proliferation through the pathways discussed above. Effects of tension on ERK, PI3K and Rho family GTPases could also contribute. These results may be relevant to the wide range of systems in which malleable matrices inhibit cell proliferation but promote differentiation (reviewed by Adams and Watt, 1993).

Conclusions and summary
Anchorage dependence of growth was described in the 1960s,
but as late as 1988 it was possible for a reviewer to write that “the molecular mechanisms of these effects are completely unknown” (Ruoslahti, 1988). Immense progress over the past decade or so has revealed that integrins both transmit direct signals and synergize with growth factor receptors to regulate the pathways that control the G1 cyclin-CDK machinery. Additionally, it is clear that a subset of integrin signals depends upon the organization and physical characteristics of the matrix and that these factors can also influence the cell cycle. Many distinct events involving multiple pathways have now been described, and it is likely that many of these events contribute to the complex networks that determine cell cycle progression. The best-characterized pathway involves cyclin D1, which many labs have found depends on both integrins and growth factors via several mechanisms to induce its expression. The role of ERK has been especially well characterized and appears to be functionally important. We suggest that the integrin-ERK-cyclin pathway may therefore serve as a paradigm for understanding how integrins combine with growth factors to control the cell cycle (see Fig. 3).

Many questions remain about the full range and mechanisms of these effects; however, two areas of greatest ignorance stand out. First, despite extensive data on pathways controlled by integrins, the proximal mechanisms by which integrins signal remain poorly defined. Second, the contribution of Rac and PI3K are very poorly understood. We eagerly await the potential to stimulate the expression of cyclin D1 mRNA. Rac and PI3K are also involved in the translation and stabilization of cyclin D1 protein, respectively.

Fig. 3. Regulation of cyclin D1. A working model depicts the cooperative effects of growth factor receptors and integrins on PI3K, ERK and Rac that regulate cyclin D1 levels. Each of these signaling components has the potential to stimulate the expression of cyclin D1 mRNA. Rac and PI3K are also involved in the translation and stabilization of cyclin D1 protein, respectively.

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