Positive regulation of cell-cell and cell-substrate adhesion by protein kinase A

John D. Whittard and Steven K. Akiyama*

Laboratory of Molecular Carcinogenesis, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC 27709, USA

*Author for correspondence (e-mail: akiyama@niehs.nih.gov)

Accepted 1 June 2001

SUMMARY

Integrin receptor activation is an important regulatory mechanism for cell-substrate and cell-cell adhesion. In this study, we explore a signaling pathway activated by mAb 12G10, an antibody that can activate β1 integrins and induce integrin-mediated cell-cell and cell-substrate adhesion. We have found that the cAMP-dependent protein kinase (PKA) is required for both mAb 12G10-induced cell-cell and cell-substrate adhesion of HT-1080 cells. Binding of mAb 12G10 to β1 integrins stimulates an increase in intracellular cAMP levels and PKA activity, and a concomitant shift in the localization of the PKA type II regulatory subunits from the cytoplasm to areas where integrins expressing the 12G10 epitope are located. MAb 12G10-induced cell-cell adhesion was mimicked by a combination of clustering β1 integrins and elevating PKA activity with Sp-adenosine-3',5'-cyclic monophosphorothioate or forskolin. We also show that two processes required for HT-1080 cell-cell adhesion, integrin clustering and F-actin polymerization are both dependent on PKA. Taken together, our data suggest that PKA plays a key role in the signaling pathway, resulting from activation of β1 integrins, and that this enzyme may be required for upregulation of cell-substrate and cell-cell adhesion.

Key words: Integrin activation, Cell-cell adhesion, cAMP, PKA, F-actin, Signal transduction

INTRODUCTION

Integrins are a large family of heterodimeric cell-surface adhesion receptors that mediate interactions of cells with both cell-surface counter-receptors and extracellular matrix ligands. In addition to mediating cell-substrate and cell-cell adhesion, integrins can regulate cellular processes such as proliferation, survival, differentiation and migration (Schwartz et al., 1995; Hynes, 1996). Although apparently devoid of any intrinsic signaling capacity, integrins are able to transduce signals bidirectionally across the plasma membrane through their ability to assemble cytoplasmic signaling complexes, which may include adaptor proteins, cytoskeletal proteins, growth factor receptors and kinases (Miyamoto et al., 1995; Schneller et al., 1997; Hemler, 1998; Howe et al., 1998; Danen et al., 1998; Giancotti and Ruoslahti, 1999; Schoenwaelder and Burridge, 1999).

Integrins can be activated by divalent cations, synthetic peptides and certain antibodies (Humphries, 1996). Recently, we described the effects of an activating anti-β1 mAb, 12G10, that can specifically and rapidly induce both cell-substrate and cell-cell adhesion of a number of different cell types (Mould et al., 1995; Whittard and Akiyama, 2001). Binding of mAb 12G10 also induced clustering of cell-surface integrins, F-actin polymerization and the preferential localization of β1 integrins expressing the 12G10 epitope at sites of cell-cell adhesion. Integrin activation-induced HT-1080 cell-cell adhesion minimally requires the interaction of activated αβ1 with nonactivated αβ1. MAb 12G10 is thought to recognize an activation-dependent epitope located close to the ligand-binding pocket of the β1 integrin (Mould et al., 1998). This epitope can be expressed by β1 integrins in the absence of ligand (Whittard and Akiyama, 2001) and may represent a naturally existing conformer of the receptor. The signaling pathways associated with activation of integrin receptors are not well characterized.

Protein phosphorylation is a pivotal mechanism in the transduction of signaling events, and the relative activities of protein kinases and protein phosphatases are highly regulated. The cAMP-dependent protein kinase (PKA) is activated as a result of interaction with cAMP, a well-studied second messenger signal classically generated through G-protein-coupled receptor stimulation of adenylyl cyclase (Gilman, 1987). The PKA holoenzyme is a tetramer consisting of two catalytic subunits and a regulatory (R) subunit dimer (Potter et al., 1978). The cAMP-PKA pathway can regulate many cellular processes such as cell metabolism (Edelman et al., 1987), proliferation (Boynton and Whitfield, 1983) and gene transcription (Montminy, 1997).

Here, we show that PKA is essential for mAb 12G10-induced HT-1080 cell-cell and cell-substrate adhesion. Binding of mAb 12G10 to β1 integrins stimulates an increase in intracellular cAMP levels and PKA activity. We also show that two processes required for HT-1080 cell-cell adhesion, that is, integrin clustering and F-actin polymerization, are both dependent on PKA. Taken together, our data suggest that PKA is a key part of a signaling pathway that is stimulated by activation of integrins and may be required for enhanced levels of cell-substrate and cell-cell adhesion.
MATERIALS AND METHODS

Monoclonal antibodies

MAb K20, directed against the human β1 integrin subunit, was obtained from Immunotech (Westbrook, ME). MAb 12G10, directed against the human β1 integrin subunit, has been described previously (Mould et al., 1995). The 12G10 mAb was directly coupled to Alexa™ 568 using the Molecular Probes protein labeling kit (Eugene, OR). Goat antibody directed against the Fc region of mouse IgG was obtained from Pierce Chemical Company (Rockford, IL). Polyclonal goat antibody to PKA type II regulatory (RII) subunits was obtained from Upstate Biotechnology (Lake Placid, NY). Alexa™ 488-coupled donkey anti-goat antibody was obtained from Molecular Probes.

Cell culture and reagents

HT-1080 human fibrosarcoma cells were obtained from American Type Culture Collection (Manassas, VA) and cultured as described (Whittard and Akiyama, 2001). Staurosporine, calphostin C, genistein, H89, myristoylated heat-stable protein kinase A inhibitor (MPKI, 14-22) peptide, myristoylated protein kinase C (PKC) α and β inhibitor (20-28) peptide, forskolin, Sp-adenosine-3′,5′-cyclic monophosphorothioate (Sp-cAMPS), 8-Bromo-cAMP, Sp-5,6-dichloro-1-β-D-ribofuranosylbenzimidazole-3′,5′-monophosphorothioate (Sp-5,6-DCl-cBiMPS) and Rp-adenosine-3′,5′-cyclic monophosphorothioate (Rp-cAMPS) were obtained from Novabiochem Corp., La Jolla, CA) and near-confluent HT-1080 cells were blocked with 10 mg/ml heat-denatured BSA (Calbiochem-Novabiochem Corp., La Jolla, CA) and then suspended in 0.5 ml of ice-cold extraction buffer (25 mM Tris-HCl, pH 7.4, 0.5 mM EDTA, 0.5 mM EGTA, 10 mM β-mercaptoethanol, 1 µg/ml leupeptin and 1 µg/ml aprotinin). Samples were homogenized with a Pellet Pestle™ homogenizer (Kimbler-Kontes, Vineland, NJ) at 4°C, and nuclei were removed by centrifugation at 14,000 g for 5 minutes at 4°C. The protein concentrations of the supernatants were determined by using the BCA protein assay (Pierce Chemical Company). PKA activity in the supernatants was measured using the Promega SignaTECT™ assay system as described (Goueli et al., 1995).

cAMP assay

Tissue culture dishes (35 mm; Becton Dickinson Labware) were coated with 1.0 ml aliquots of 10 µg/ml poly-D-lysine overnight at 4°C. Dishes were blocked and near-confluent cells were harvested as described for PKA assays. Cells (1.0 ml) were added to dishes with or without antibodies for 20 minutes at 37°C. Cells were washed three times with ice-cold PBS and extracted with 0.5 ml 5% (w/v) trichloroacetic acid for 5 minutes. Supernatant was combined with 9 ml 5% trichloroacetic acid and homogenized with a Pellet Pestle™ homogenizer (Kimbler-Kontes). CAMP in the supernatant was quantitated using the Biomedical Technologies Inc. enzyme immunoassay system kit according to the manufacturer’s instructions.

Immunofluorescence microscopy

Immunofluorescence localization was performed as described (Whittard and Akiyama, 2001). F-actin was stained with 0.5 units/ml Alexa™ 488 phalloidin, the PKA RII subunits were localized using 5 µg/ml polyclonal antibody and β1 integrins were localized using 10 µg/ml mAb 12G10 directly labeled with Alexa 568™, all for 30 minutes at room temperature. The antibody directed against the PKA RII subunits was detected using Alexa™ 488-coupled donkey anti-goat IgG for 30 minutes at room temperature.

RESULTS

Integrin mediated cell-cell adhesion is dependent on PKA

It has been previously shown that cell-cell adhesion results from activation of β1-integrins by mAb 12G10 (Whittard and Akiyama, 2001). As shown in Fig. 1A, 100 nM staurosporine significantly inhibited mAb 12G10-induced HT-1080 cell-cell adhesion (P<0.01). At this concentration, staurosporine could inhibit a variety of protein kinases including PKC, PKA, protein kinase G and tyrosine kinases (Ruegg and Burgess, 1989). Neither calphostin C, a PKC inhibitor, nor genistein, a tyrosine kinase inhibitor, were able to inhibit mAb 12G10-dependent cell-cell adhesion (Fig. 1A), apparently ruling out a role for these kinase families in mAb 12G10-induced cell-cell adhesion. By contrast, H89 and MPKI peptide, two specific PKA antagonists, each inhibited HT-1080 cell-cell adhesion in a dose-dependent manner (Fig. 1B,C). The increase in cell-cell adhesion when cells were treated with mAb 12G10 (Fig. 1D,E) was completely inhibited with 20 µM MPKI peptide (Fig. 1C,F). In a control experiment, 20 µM of a myristoylated PKC inhibitor peptide of similar molecular weight to the MPKI peptide had no effect on mAb 12G10-activated cell-cell adhesion (data not shown). This result apparently rules out the possibility of nonspecific inhibition of cell-cell adhesion.
caused by the presence of myristoylated peptide. Taken together, these results suggest that PKA, but not PKC or tyrosine kinases, participates in a signaling pathway stimulated by mAb 12G10 that results in the induction of cell-cell adhesion.

Cell-substrate adhesion can also be enhanced as a consequence of mAb 12G10-activation of β1 integrins (Mould et al., 1995; Whittard and Akiyama, 2001). Therefore, we examined whether PKA-mediated signaling was also required for the increased cell-substrate adhesion resulting from integrin activation with mAb 12G10. Treatment of cells with mAb 12G10 resulted in increased cell attachment to collagen type IV above basal levels (Fig. 2; P<0.01). Basal cell adhesion to collagen type IV was partially inhibited (40%; P<0.01) when cells were pretreated with 20 μM MPKI peptide and almost completely inhibited (80%; P<0.01) when pretreated with 2 μM calphostin C. However, there was only a very minor inhibition (15%; P<0.01) of attachment to collagen type IV when mAb 12G10-activated cells were pretreated with 2 μM calphostin C. By contrast, pre-treating these cells with 20 μM MPKI peptide resulted in a marked decrease (50%; P<0.01) in activated HT-1080 cell attachment to collagen type IV. Therefore, activated cell adhesion to collagen type IV was largely resistant to the inhibitory effects of calphostin C, but was inhibited by the MPKI peptide. These results suggest that the enhanced levels of cell-substrate adhesion observed following integrin activation may also require PKA-dependent phosphorylation.

Activating integrins with mAb 12G10 leads to an increase in intracellular cAMP levels and PKA activity

Our findings so far suggest that mAb 12G10 may induce cell-cell and cell-substrate adhesions by activating a PKA-dependent signaling pathway. In order to test this hypothesis directly, we investigated whether mAb 12G10 binding to integrins was able to stimulate an increase in intracellular cAMP levels and also PKA enzymatic activity. Treatment of cells with either 10 μg/ml mAb 12G10 or 5 μM forskolin, a potent activator of adenyl cyclase, resulted in a threefold increase in the levels of cAMP above either basal level or the level observed with the nonactivating anti-β1 mAb K20 (Fig. 3A; P<0.01). Similarly, treatment of cells with mAb 12G10 also resulted in increased levels of PKA enzymatic activity above the basal level (Fig. 3B; P<0.05). The nonactivating anti-β1 mAb K20 had no effect on PKA enzymatic activity. In fact, the increase in PKA activity resulting from activation of integrins with mAb 12G10 was similar to that observed with 10 μM Sp-cAMPS, a cAMP analogue that activates PKA. In control experiments, another activating anti-β1 mAb, TS2/16, had no detectable effect on PKA enzymatic activity (data not shown). These results demonstrate that activation of β1 integrins with mAb 12G10 stimulates an increase in intracellular levels of both cAMP and PKA enzymatic activity.

Integrin clustering and an increase in PKA activity are both required to induce cell-cell adhesion

Treatment of HT-1080 cells with Rp-cAMPS, a cAMP analogue that inhibits PKA activity, inhibited mAb 12G10-
Fig. 3. Activation of β1-integrins with mAb 12G10 causes an increase in intracellular cAMP levels and PKA activity. (A) HT-1080 cells were incubated with 10 μg/ml anti-β1 integrin antibodies or 5 μM forskolin and assayed for cAMP as described in Materials and Methods. Asterisk indicates *P<0.01 vs. no treatment. Values shown are mean±s.e.m. (n=4). (B) HT-1080 cells were incubated with 10 μg/ml anti-β1 integrin antibodies or 10 μM Sp-cAMPS and assayed for PKA activity using the biotinylated kemptide peptide substrate as described in Materials and Methods. Asterisk indicates *P<0.05 vs. no treatment. Values shown are mean±s.d. (n=2).

Fig. 4. MAb 12G10-induced cell-cell adhesion is dependent on cAMP. The effect of Sp-cAMPS on HT-1080 cell-cell adhesion stimulated with 10 μg/ml 12G10. The dotted lines represent the level of basal HT-1080 cell-cell adhesion±s.d. (n=8). CCAI was scored and defined as described in Materials and Methods.

PKA activity alone would be sufficient to induce cell-cell adhesion, HT-1080 cells were treated with 10 μM Sp-cAMPS and assayed for cell-cell adhesion. There was a small, but significant, increase in the level of cell-cell adhesion (Fig. 5A; *P<0.01 vs. no treatment). Similar results were found with two other cAMP analogues that activate PKA (8-Bromo-cAMP and SP-5,6-DCI-cBimPS; data not shown). However, none of the cAMP analogues activated cell-cell adhesion to levels attainable with mAb 12G10. Using mAb 12G10 at a suboptimal concentration of 0.3 μg/ml (Whittard and Akiyama, 2001) resulted in a twofold increase in cell-cell adhesion above basal levels (Fig. 5A; *P<0.01 vs. no treatment). Interestingly, a combination of 0.3 μg/ml mAb 12G10 and 10 μM Sp-cAMPS resulted in a level of cell-cell adhesion that was greater than the sum increase of either treatment alone (Fig. 5A; *P<0.01). These results suggest that sub-optimal concentration of mAb 12G10 may act synergistically with Sp-cAMPS to induce near maximal levels of HT-1080 cell-cell adhesion.

The results shown in Figs 4 and 5A suggest that an increase in PKA activity plus at least one other process stimulated by mAb 1210 may be required for integrin activation to lead to cell-cell adhesion. We have previously shown that mAb 12G10 stimulates integrin clustering, but integrin clustering alone cannot induce cell-cell adhesion (Whittard and Akiyama, 2001). We therefore examined whether an increase in PKA...
activity in conjunction with integrin clustering would mimic the effects of 12G10 activation and induce cell-cell adhesion. When β₁ integrins were clustered using mAb K20 and anti-mouse IgG, as described in Materials and Methods, there was a small increase in the level of HT-1080 cell-cell adhesion (Fig. 5B). A similar, small increase in cell-cell adhesion was observed when cells were treated with 10 μM Sp-cAMPS (Fig. 5B). However, a combination of integrin clustering and 10 μM Sp-cAMPS resulted in a level of cell-cell adhesion similar to that observed with 10 μg/ml mAb 12G10. Essentially identical results were observed when β₁ integrins were clustered in the presence of 5 μM forskolin (data not shown). In control experiments, the clustering of β₁ integrins alone was not sufficient to induce an increase in intracellular cAMP levels (data not shown). Thus, both the clustering of β₁ integrins and an increase in PKA activity are required to induce integrin-mediated cell-cell adhesion.

Inhibiting PKA blocks F-actin polymerization and integrin clustering induced as a result of integrin activation

Previously, we showed that mAb 12G10-induced cell-cell adhesion may require both integrin clustering and polymerization of F-actin (Whittard and Akiyama, 2001). We therefore decided to explore whether inhibiting PKA would affect either of these processes. On nonactivated HT-1080 cells, integrins expressing the 12G10 epitope were uniformly distributed on the surface of cells (Fig. 6, top panels). There was also a small amount of F-actin around the periphery of HT-1080 cells. By contrast, cells treated with mAb 12G10 exhibited clustering of integrins expressing the 12G10 epitope and also increased levels of F-actin polymerization in single cells (Fig. 6, middle panels). Locations of integrin clustering

Fig. 6. Integrin clustering and F-actin polymerization are both dependent on PKA. HT-1080 cells were treated with 20 μM MPKI peptide for 30 minutes and plated on poly-D-lysine coated cover slips for another 20 minutes in the presence of no antibody or 10 μg/ml 12G10 directly labeled with Alexa™ 568. F-actin was visualized with fluorescently labeled phalloidin (green) and β₁ integrins were visualized with Alexa™ 568-labeled 12G10 (red). Fluorescent images were combined to determine colocalization (yellow). Bar, 10 μm.

Fig. 7. Colocalization of PKA RII subunits with integrins expressing the 12G10 epitope following integrin activation. HT-1080 cells were treated with 10 μM Sp-cAMPS for 20 minutes, and plated on poly-D-lysine coated cover slips for another 20 minutes in the presence of no antibody or 10 μg/ml of 12G10 directly labeled with Alexa™ 568. PKA RII subunits were visualized by indirect immunofluorescence using fluorescently labeled donkey anti-goat IgG antibody (green) and β₁ integrins were visualized with Alexa™ 568-labeled 12G10 (red). Fluorescent images were combined to determine colocalization (yellow). All bars, 10 μm.
coincided with sites of increased F-actin. MAb 12G10-induced integrin clustering and F-actin polymerization were both inhibited when cells were treated with 10 μM MPKI peptide prior to the addition of mAb 12G10 (Fig. 6, bottom panels). These results suggest the hypothesis that the mAb 12G10-induced increase of PKA activity was required for integrin clustering and F-actin polymerization, and thus was likely to be upstream of both processes.

**PKA RII subunits colocalize with integrins expressing the 12G10 epitope following integrin activation**

The two R subunits (RI and RII) of PKA are differentially localized inside cells; RIIα and RIIβ subunits are mainly associated with cellular structures and organelles, whereas RIα and RIβ subunits are localized predominantly in the cytoplasm (Scott, 1991). The compartmentalization of the multithreaded PKA enzyme, usually through interactions with A-kinase anchoring proteins (AKAPs), is thought to be an important mechanism for controlling enzymatic specificity (Colledge and Scott, 1999; Edwards and Scott, 2000; Diviani and Scott, 2001). We may elucidate valuable insight into the phosphorylation target(s) of PKA by determining the localization of the PKA RII subunits following integrin activation with mAb 12G10. As previously shown, integrins expressing the 12G10 epitope were uniformly distributed on the surfaces of nonactivated cells (Fig. 7, upper panels). In nonactivated HT-1080 cells, the PKA RII subunits were localized primarily throughout the cytoplasm, with only a small amount around the cell surface (Fig. 7, upper panels). However, there was a dramatic change in the localization of the PKA RII subunits following activation of integrins with mAb 12G10. Instead of being localized mainly in the cytoplasm, the PKA RII subunits colocalized with integrins expressing the 12G10 epitope at sites of cell-cell contact (Fig. 7, middle panels) and areas of integrin clustering in single cells (Fig. 7, bottom panels). These results suggest that activation of integrins with mAb 12G10 leads to a translocation of the PKA RII subunits from the cytoplasm to areas where integrins expressing the 12G10 epitope are located. These results also suggest the possibility that the target(s) of PKA phosphorylation may be positioned in close proximity to integrins expressing the 12G10 epitope.

**DISCUSSION**

We have demonstrated that mAb 12G10-activation of β1 integrins stimulates an increase in intracellular levels of cAMP and PKA activity. This increase in PKA activity is necessary for the enhanced levels of cell-cell and cell-substrate adhesion observed following integrin activation but is not, by itself, sufficient. However, integrin clustering in addition to stimulation of the cAMP-PKA pathway is sufficient to mimic monoclonal antibody activation of integrin-mediated cell-cell adhesion. We also find that integrin activation results in a translocation of PKA RII subunits from the cytoplasm to sites where the activated integrins are clustered.

There is increasing evidence suggesting that integrins can regulate the activation of a number of signaling pathways inside adherent cells, including the MAP kinase cascade, Rho family of guanosine triphosphatases, tyrosine kinases, phosphatidylinositol 3-kinase and Jun amino-terminal kinase pathways (Giancotti and Ruoslahti, 1999). Results presented here demonstrate that the cAMP-PKA pathway can be stimulated in response to activating β1 integrins with mAb 12G10. Recent work has shown that mechanically stressed ligand-bound β1 integrins can activate the cAMP-PKA pathway in a G-protein-dependent manner (Meyer et al., 2000). The cAMP-PKA pathway can also be stimulated upon adhesion of endothelial cells to extracellular matrix proteins (Fong and Ingber, 1996) and transiently activated following detachment of 3T3 fibroblasts (Howe and Juliano, 2000).

The mechanism by which activation of β1 integrins can lead to an increase in the cAMP-PKA pathway is currently unknown. The simplest hypotheses are that an increase in PKA activity may result from the activation of adenylyl cyclase or, alternatively, activation results in inhibition of phosphodiesterases. Interestingly, elevation of intracellular free Ca2+ levels have been observed following integrin ligation (Schwartz, 1993), and these increases could activate Ca2+-sensitive adenylyl cyclases (Watson et al., 2000). However, adenylyl cyclases are more typically activated as a result of heterotrimeric Gαq protein coupling to G-protein-coupled receptors at the cytoplasmic face of the plasma membrane (Gudermann et al., 1997). A recent study has shown that an αvβ3 integrin-associating protein (CD47) complex could couple with, and consequently stimulate, the inhibitory trimeric Gαi protein, leading to a rapid decrease in cAMP (Frazier et al., 1999). We hypothesize that an activated β1-integrin may be involved in a protein complex that could couple with the trimeric Gαi protein, and thereby stimulate an increase in the cAMP-PKA pathway.

The data reported here point to the PKA enzyme as being a crucial component of a signaling pathway(s), stimulated by activation of integrins, and that may be required for enhanced levels of cell-substrate and cell-cell adhesion. In the case of HT-1080 cells, it appears that an increase in PKA activity is required for integrin-activation-dependent cell-substrate and cell-cell adhesion. Several lines of evidence, however, suggest that integrin-mediated events can be negatively regulated by an increase in the cAMP-PKA pathway. For example, an increase in cAMP has been shown to alter cell morphology, induce the disassembly of stress fibers and focal contacts, or inhibit cell spreading and migration of certain cell types (Lamb et al., 1988; Lampugnani et al., 1990; Glass and Kreisberg, 1993; Leven, 1995). Thus, it appears that an increase in PKA activity may elicit different responses depending on how far the adhesion process has advanced, and on the cell type.

The target(s) of PKA phosphorylation that play a role in the regulation of cell-cell adhesion are unknown. PKA is a multisubstrate enzyme that can phosphorylate a broad spectrum of protein substrates (Walsh and van Patten, 1994). Conversely, PKA phosphorylation can also inhibit the function of several signaling molecules, including RhoA (Lang et al., 1996; Dong et al., 1998), activin (Ohta et al., 1987), p21-activated kinase (Howe and Juliano, 2000) and paxillin (Han and Rubin, 1996). Therefore, the involvement of PKA in the process of cell-cell adhesion may be a consequence of PKA inhibiting certain signaling molecules such as Rho, thereby leading to the upregulation of other proteins such as Rac (Sander et al., 1999). Interestingly, we observe two processes
following treatment with mAb 12G10 that are typically indicative of Rac activation: F-actin polymerization and lamellipodia formation (Whittard and Akikyama, 2001).

Immunocytochemical studies suggest that activation of β1 integrins may regulate the localization of the PKA RII subunits in HT-1080 cells. Following integrin activation, the RII subunits appear to translocate from the cytoplasm to areas where integrins expressing the 12G10 epitope reside. This result suggests that the target(s) of PKA phosphorylation may be positioned in close proximity to integrins expressing the 12G10 epitope. A change in PKA RII subunit localization may be dependent upon AKAPs, as these proteins have been shown to bind with high affinity to PKA RII subunits (Carr et al., 1991). Determining which, if any, of the AKAPs are involved in the process of cell-cell adhesion may provide valuable information in identifying downstream pathways targeted by PKA phosphorylation.

In conclusion, our data suggest that PKA plays a key role in the signaling pathway resulting from activation of integrins and that PKA may be required for enhanced levels of cell-substrate and cell-cell adhesion. To the best of our knowledge, this may be the first demonstration that an integrin-mediated increase in the activity of the cAMP-PKA pathway can positively regulate tumor-cell-adhesive interactions. There are many questions still to be answered to provide an understanding of the mechanisms by which integrin-mediated adhesive events control PKA activity, and conversely, how those events are regulated by the cAMP-PKA pathway. These answers could lead to the identification and characterization of molecular mechanisms of physiological and pathological processes that may require homotypic integrin interactions. For example, these adhesive interactions could be especially important during embryonic development and the subsequent maintenance of body organs. Furthermore, pathological processes that require both the formation and disruption of cell-cell contacts, such as tumor metastasis, could also be regulated by homotypic integrin interactions.

We thank J. M. Reece for technical assistance with confocal microscopy, and E. Murphy and J. D. Roberts for critical review of the manuscript. We also thank F. Ribeiro-Neto for his helpful discussion and critical review of the manuscript, and J. K. Haseman for assistance with the statistics.

REFERENCES


