Epidermal Growth Factor Modulates Tyrosine Phosphorylation of p130Cas

IN Volvement of Phosphatidylinositol 3'-Kinase and Actin Cytoskeleton*

(Received for publication, July 2, 1997, and in revised form, July 28, 1997)

Marja Ojaniemi‡ and Kristiina Vuori§

From the La Jolla Cancer Research Center, The Burnham Institute, La Jolla, California 92037

Epidermal growth factor (EGF) treatment of Rat-1 cells expressing human EGF receptor results in the modification of the tyrosine phosphorylation of the p130 Crk-associated substrate (Cas), a novel signaling molecule residing in focal adhesions. At low, mitogenic concentrations (<10 ng/ml), EGF treatment induced a rapid and transient tyrosine phosphorylation of Cas and promoted the formation of a Cas-adapter protein Crk complex in intact cells. The increase in tyrosine phosphorylation of Cas paralleled an increase in the cellular content of actin stress fibers and occurred via a pathway that depended on the integrity of the cytoskeleton. Further, phosphatidylinositol 3'-kinase activity was found to be required for the EGF-stimulated Cas phosphorylation and actin polymerization. At high concentrations (>30 ng/ml), EGF treatment resulted in the tyrosine dephosphorylation of Cas in a time-dependent manner with a concomitant decrease in the length and number of actin stress fibers. Thus, Cas exhibits an unusual bell-shaped dose-response curve in response to EGF stimulation. These results demonstrate a novel signaling role for EGF in inducing changes in tyrosine phosphorylation of Cas and Cas-Crk complex formation and suggest that Cas could be a signaling component in EGF-mediated signal transduction.

The binding of growth factors to their receptors results in receptor dimerization and subsequent transphosphorylation of specific tyrosine residues in the cytoplasmic domains of the receptors. The phosphorylated residues in turn serve as attachment sites for cellular Src homology 2 (SH2) domain-containing signaling proteins; formation of these signal transduction complexes coordinates the multiple intracellular programs that initiate various changes in cell proliferation and differentiation (1). In addition to the mitogenic effects, many growth factors are found to be required for the EGF-stimulated Cas phosphorylation and actin polymerization. At high concentrations of PDGF (30 ng/ml) results in tyrosine dephosphorylation of the focal adhesion proteins with a concomitant decrease in the length and number of actin stress fibers (12, 14, 16–18). These data have established a novel role for EGF stimulation of cells with epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and insulin, the cells exhibit rapid membrane ruffling; this is brought about by a reorganization of the actin microfilament system, which includes extensive local actin polymerization in the cell cortex (2). A cascade of small GTP-binding proteins of the Rho family has been implicated in mitogen-stimulated changes in the actin cytoskeleton. In particular, Rac1 is responsible for membrane ruffling and lamellipodia formation, whereas activation of RhoA mediates the assembly of focal adhesions and stress fibers at a later time point following growth factor stimulation (2–5). Recent experiments demonstrate that activation of phosphatidylinositol 3'-kinase (PI 3-kinase) upon PDGF stimulation is required for Rac1 activation and subsequent membrane ruffling (6–9).

The mitogen-induced changes in actin cytoskeleton are accompanied by dramatic changes in tyrosine phosphorylation of several proteins present in focal adhesions. Focal adhesions are regions of a cell in direct contact with the extracellular matrix, providing anchorage sites for actin stress fibers and forming a link between the extracellular matrix and the actin cytoskeleton via the integrin family of cell surface receptors (10, 11). Stimulation of cells with low, mitogenic concentrations of PDGF (1–10 ng/ml) results in PI 3-kinase activation, membrane ruffle formation, and simultaneous tyrosine phosphorylation of focal adhesion proteins (12, 14, 16–19). These data have established a novel role for PDGF and insulin in modulating the signaling molecules residing in the focal adhesions.

p130Cas (Cas, Crk-associated substrate) is a novel, 130-kDa focal adhesion protein that was originally identified as a prominent tyrosine-phosphorylated protein in v-Src- and v-Crk-transformed cells. Molecular cloning of Cas revealed a docking protein that contains an SH3 domain, proline-rich regions, and a cluster of 15 putative SH2-binding motifs (19). The unique structure of Cas suggests a role in assembling multiprotein signaling complexes. Indeed, Cas has been reported to become tyrosine-phosphorylated in response to a number of different stimuli, many of which affect the assembly of focal adhesions and actin stress fibers. These stimuli include integrin-mediated cell adhesion (20–22), ligation of the B-cell receptor and interleukin-8 receptor (23, 24), and stimulation of cells with nerve growth factor (25), bombesin and other neuropeptides, phorbol esters, bioactive lipids, and PDGF (14). Following tyrosine phosphorylation, Cas interacts with a number of SH2-containing signaling molecules, such as the adapter proteins...
Crk and Nck, possibly recruiting these molecules to focal adhesions (26, 27).

Previous studies have demonstrated that stimulation of cells with EGF results in dramatic changes in cell morphology (28); in the present study, we report that stimulation of cells with EGF induces changes in actin cytoskeleton with a concomitant modulation of the tyrosine phosphorylation of Cas. Similar to the PDGF response (14), we found that the phosphorylation of Cas exhibits an unusual bell-shaped dose-response curve upon EGF stimulation and that the tyrosine phosphorylation of Cas by low concentrations of EGF requires the integrity of the actin cytoskeleton and PI 3-kinase activity. Furthermore, low concentrations of EGF promote the formation of a Cas-Crk complex in intact cells. These results identify a novel signaling role for EGF and support the notion that Cas could play a role in mitogen-mediated signal transduction.

EXPERIMENTAL PROCEDURES

Reagents—Dulbecco’s modified Eagle’s medium was supplied by Mediatech (Herndon, VA). Fetal calf serum was from Tissue Culture Biologicals (Tulare, CA), and Glutamine Pen-Strep was from Irvine Scientific (Santa Ana, CA). EGF was from Life Technologies, Inc. PI 3-kinase inhibitors wortmannin and LY 494502 were from Calbiochem. Polyclonal anti-Cas antibody (C-20) was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), and monoclonal anti-p120 Ras-GAP antibody and horseradish peroxidase-conjugated anti-phosphotyrosine antibody py20 were from Transduction Laboratories (Lexington, KY). Polyclonal anti-Crk antibody was from Dr. Michiyuki Matsuda (National Institute of Health, Tokyo, Japan). All other reagents were acquired from Sigma.

Cell Culture and Cell Stimulation—Rat-1 cells expressing human EGF receptor (RatER cells; Ref. 29), A431 cells, and wild-type Rat-1 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 50 units/ml penicillin, and 50 μg/ml streptomycin. Cells were grown into 90% confluency as monolayers and serum-starved for 36 h prior to stimulation with or without EGF in the presence or absence of various inhibitors as indicated in the figure legends.

Preparations of Cell Lysates, Immunoprecipitations, and Immunoblotting—Following stimulation, cells were washed with ice-cold phosphate-buffered saline (PBS) and lysed in modified radioimmune precipitation buffer (50 mM Hepes, pH 7.5, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 0.1% SDS, 1% sodium deoxycholate, 100 mM NaF, 0.5 mM Na3VO4, 1 mM EGTA, 0.1 unit/ml aprotinin, 10 μg/ml leupeptin, and 4 μg/ml pepstatin A). Immunoprecipitations and immunoblot analysis were carried out as described earlier (21).

Fluorescent Labeling of Filamentous Actin—Confuent cultures of RatER cells following treatments indicated in the figure legends were washed twice with PBS and then fixed in 4% paraformaldehyde in PBS for 10 min at room temperature. The fixed cells were washed twice with PBS and extracted with a solution of 20% acetone for 3 min. Immunofluorescent labeling of filamentous actin was carried out by incubation for 30 min at room temperature with 0.1 mg/ml of carboxytetramethylrhodamine isothiocyanate (TRITC)-phalloidin (Sigma). Following incubation with the fluorochrome-conjugated phalloidin, the cells were rinsed three times with PBS, and the samples were mounted for microscopy.

Assay for DNA Synthesis—Cells (105/well) were seeded in a 96-well plate and made quiescent by starvation for 36 h. The quiescent cells were grown into 90% confluency as monolayers and serum-starved for 36 h prior to stimulation with or without EGF in the presence or absence of various inhibitors as indicated in the figure legends.

RESULTS

Tyrosine Phosphorylation of Cas Exhibits a Bell-shaped Dose Response upon EGF Stimulation—Previous studies have reported that a recently identified focal adhesion protein Cas provides an important integration site for various extracellular signals including integrin receptor family members, G-protein coupled receptors, and both receptor and nonreceptor tyrosine kinases (see the Introduction). These signals appear to increase Cas tyrosine phosphorylation and enhance actin polymerization. Stimulation of cells with EGF results in dramatic changes in cell morphology and reorganization of the actin microfilament system (28). To investigate the putative role of EGF in the regulation of Cas-mediated signaling, we examined the effect of EGF on tyrosine phosphorylation of Cas in various EGF-responsive cell lines. Unless otherwise indicated, the results shown in the figures are those obtained with RatER cells (2.5 × 105 receptors/cell; Ref. 29). In addition, epidermoid carcinoma A431 cells (>106 receptors/cell; Ref. 28) and wild-type Rat-1 cells were studied, and similar results were obtained. Quiescent cultures of RatER cells were incubated with various concentrations of EGF for 5 min, lysed, and immunoprecipitated with an anti-Cas antibody. This antibody recognizes the extreme C terminus of Cas and does not cross-react with Efs/Sin or HEF-1/Cas-L, which are proteins closely related to Cas (31–34). The immunoprecipitates were analyzed by SDS-polyacrylamide gel electrophoresis followed by immunoblotting with anti-phosphotyrosine antibodies. Compared with the unstimulated cells (Fig. 1A, lane 1), there was a detectable increase in Cas tyrosine phosphorylation following treatment with low concentrations of EGF (0.5–5 ng/ml, lanes 2–5). In contrast, the phosphorylation content of Cas decreased sharply at high concentrations of EGF. Maximal reduction of tyrosine phosphorylation took place at 60 ng/ml of EGF and was further affected by treatment with higher concentrations of EGF (lanes 9–11). The changes in the tyrosine phosphorylation of Cas occurred with no alteration in the amount of Cas protein immunoprecipitated under these conditions (Fig. 1A, lower panel). As a comparison, we studied tyrosine phosphorylation of phospholipase C-γ and p120 Ras-GAP, which are two previously identified substrates for the EGF receptor kinase (35, 36). Tyrosine phosphorylation of these proteins increased in a dose-dependent manner in response to EGF stimulation, with no bell-shaped dose response curve; results for p120 Ras-GAP are shown in Fig. 1C.

To further characterize the effect of EGF on tyrosine phosphorylation of Cas, we examined the time dependence of Cas phosphorylation status following EGF treatment. Low concentrations of EGF stimulated a very rapid and transient tyrosine phosphorylation of Cas; an increase in tyrosine phosphorylation of Cas could be detected as early as 30–60 s after the addition of 2 ng/ml of EGF, reaching a maximum at 1–5 min. Thereafter, Cas tyrosine phosphorylation declined and remained above or at base-line levels for >4 h (Fig. 2A, top). At high EGF concentrations (80 ng/ml), Cas tyrosine dephosphorylation was initially detected by 1 min (Fig. 2B, top). There was a progressive decline in Cas phosphorylation that reached a maximum at 5–10 min of EGF treatment. The EGF-mediated decrease in Cas phosphorylation was transient and began to recover 30 min subsequent to the addition of 80 ng/ml EGF. Following 60 min of EGF treatment, the tyrosine phosphorylation state of Cas was similar to that observed for the unstimulated control cells (Fig. 2B, compare lanes 1 and 7). During the periods of EGF treatment, there was no significant alteration in the amount of immunoprecipitated Cas (Fig. 2, A and B, bottom parts).

Effect of EGF on the Actin Cytoskeleton—It has been well documented that the formation of actin stress fibers parallels focal adhesion formation and is accompanied by increased tyrosine phosphorylation of focal adhesion proteins (for a review, see Ref. 37). Tyrosine dephosphorylation of focal adhesion proteins in response to insulin treatment in turn parallels a decrease in the cellular content of actin stress fibers (18). We next examined the effect of low and high concentrations of EGF on the organization of actin in quiescent cultures of RatER cells. The cells were treated with either 2 or 80 ng/ml of EGF for 5 min and then fixed and stained with TRITC-conjugated phal-
loidin. Treatment of cells with a low concentration of EGF (2 ng/ml) resulted in a characteristic membrane ruffling (Fig. 3B) and increased polymerization of actin (Fig. 3C). In contrast, treatment with a high concentration of EGF (80 ng/ml) for 5 min caused a dramatic disruption of actin stress fibers (Fig. 3D). Maximum effects on the actin cytoskeleton by low and high concentrations of EGF were observed after 5 min of EGF exposure. The changes in the number and length of actin stress fibers were followed by a recovery to normal levels in a time-dependent manner; restoration of the actin stress fiber network (as in Fig. 3A) was apparent within 30–60 min (not shown). Immunostaining for vinculin, which is a marker of focal adhesions, indicated the presence of focal adhesion plaques in cells treated with a low concentration of EGF. In contrast, a high concentration of EGF stimulated the disruption of vinculin-containing focal adhesions (not shown). These results indicate that at low concentrations EGF stimulates actin polymerization and Cas phosphorylation. High concentrations of EGF in turn cause disruption of the actin cytoskeleton and focal adhesions; these pronounced effects on the actin cytoskeleton are accompanied by a reduction in the tyrosine phosphorylation of Cas.

Integrity of the Actin Cytoskeleton and PI 3-Kinase Activity Are Necessary for EGF-induced Tyrosine Phosphorylation of Cas—Given the close correlation between actin polymerization and Cas phosphorylation upon cell stimulation with low concentrations of EGF, we next examined whether disruption of the actin cytoskeleton could interfere with the increase in Cas tyrosine phosphorylation by EGF. Experiments were performed using cells pretreated for 2 h with various concentrations of cytochalasin D. Treatment of cells with cytochalasin D prevented Cas phosphorylation on tyrosine in response to 2...
ng/ml of EGF in a dose-dependent manner (Fig. 4A); tyrosine phosphorylation of Cas was completely blocked using a concentration of cytochalasin D (2.0 \mu\text{M}) found to depolymerize the network of actin filaments in RatER cells (Fig. 3E). Cytochalasin D treatment had no effect on the tyrosine phosphorylation of p120 Ras-GAP at any of the EGF concentrations that stimulated p120 Ras-GAP phosphorylation; the effect on 20 ng/ml of EGF is shown in Fig. 4B. Thus, EGF-stimulated tyrosine phosphorylation of Cas, but not of the previously identified EGF receptor kinase substrate p120 Ras-GAP, is critically dependent on the integrity of the actin cytoskeleton.

It has recently been shown that activation of PI 3-kinase is required for PDGF-stimulated membrane ruffling and tyrosine phosphorylation of focal adhesion proteins (6–9, 12–14). PI 3-kinase is known to become activated upon EGF treatment (36), and we therefore examined the potential role of PI 3-kinase in EGF-stimulated tyrosine phosphorylation of Cas in RatER cells. Quiescent cells were preincubated for 2 h with increasing concentrations of LY294002, which has been identified as a specific inhibitor of PI 3-kinase with an IC_{50} of 1–10 \mu\text{M} (39). Following the pretreatment, the cells were stimulated with 2 ng/ml of EGF for 5 min. As shown in Fig. 4C, pretreatment with LY294002 inhibited Cas tyrosine phosphorylation induced by EGF in a dose-dependent fashion. Maximum inhibition was achieved with 20 \mu\text{M} of LY294002; at the same concentration, LY294002 inhibited actin cytoskeleton reorganization in EGF-treated RatER cells (Fig. 3F). To substantiate the results obtained with LY294002, we examined a structurally unrelated compound, wortmannin, which inhibits PI 3-kinase by a distinct mechanism. Wortmannin is highly specific for PI 3-kinase, although at concentrations above 1 \mu\text{M} it has been reported to inhibit myosin light chain kinase (40, 41). Pretreatment of cells with wortmannin also induced a dose-dependent inhibition of the tyrosine phosphorylation of Cas in response to 2 ng/ml EGF. At 50 \mu\text{M}, wortmannin inhibited EGF-stimulated Cas phosphorylation by >90\% (not shown). Together, these results suggest that PI 3-kinase lies upstream in the signal transduction pathway linking the EGF receptor to tyrosine phosphorylation of Cas.

**EGF Induces the Formation of a Cas-Crk Complex—** Cas has a cluster of 15 potential SH2-binding motifs, nine of which conform to the expected binding site for the SH2-domain of the adapter protein Crk (19). Indeed, it has recently been demonstrated that a number of agonists that induce Cas phosphorylation also induce Cas-Crk complex formation (14, 26). Consequently, we examined whether EGF-induced tyrosine phosphorylation of Cas could lead to the formation of a complex between Cas and Crk in intact RatER cells. Immunoblotting of anti-Cas immunoprecipitates revealed that low concentrations of EGF (2 ng/ml) stimulated an association of Cas with Crk (Fig. 5). Similarly, coimmunoprecipitation of Cas with Crk was detected when immunoprecipitation was carried out with anti-Crk antibodies and immunoblotting with anti-Cas antibodies (not shown). To assess whether the complex formation between Cas and Crk depended on the integrity of the actin cytoskeleton, quiescent RatER cells were pretreated for 2 h with 2.0 \mu\text{M} cytochalasin D and then stimulated with 2 ng/ml EGF for 5 min. Fig. 5 shows that treatment of cells with cytochalasin D, at a concentration shown in Fig. 4A to inhibit Cas tyrosine phosphorylation, prevented the association of Cas with Crk induced by EGF. As could be expected, high concentrations of EGF (80 ng/ml), which stimulate dephosphorylation of Cas on tyrosine (Figs. 1 and 2), did not induce Cas-Crk interaction (Fig. 5).

**Concentrations of EGF That Stimulate Cas Phosphorylation Are Mitogenic—** In parallel with the analysis of tyrosine phosphorylation of Cas and Cas-Crk complex formation, RatER cells were exposed to various concentrations of EGF and used to assess DNA synthesis (Fig. 6). Maximum \[^{3}\text{H}\text{]thymidine incorporation was achieved at an EGF concentration of 40–80 ng/ml with a half-maximal effect at 5–10 ng/ml. Similar results have been previously reported by others using different cell lines (42, 43). Similar to earlier observations (42, 43), high EGF...
concentrations (160 ng/ml) resulted in lower levels of [³H]thymidine incorporation, as indicated by the decline in the dose-response curve. We observed a clear induction in [³H]thymidine incorporation at EGF concentrations as low as 1.25 ng/ml. Tyrosine phosphorylation of Cas and Cas-Crk association therefore appears to occur at concentrations of EGF sufficient for inducing mitogenesis.

**DISCUSSION**

Although the exact role of p130Cas (Cas) in growth factor signaling has not been elucidated to date, it appears to be an important integration point for multiple signaling pathways. In the present report, we demonstrate that in contrast to many growth factors, EGF stimulation of fibroblasts results in an unusual bell-shaped dose-response curve of tyrosine phosphorylation of Cas. The changes in tyrosine phosphorylation of Cas paralleled EGF-induced changes in the polymerization content of actin and were found to result in changes in the complex formation between Cas and the adapter protein Crk. These results suggest a novel function for Cas as a signaling component in EGF-mediated signal transduction.

One of the most striking findings reported here is that the tyrosine phosphorylation of Cas in response to low concentrations of EGF is dramatically different from that induced by high concentrations of the growth factor; low concentrations (0.5–5 ng/ml) of EGF resulted in increased tyrosine phosphorylation of Cas, whereas higher concentrations either had no effect or induced a marked dephosphorylation of Cas (concentrations >30 ng/ml EGF). This bell-shaped dose-response curve was observed in three different cell lines (RatER, A431, and Rat-1 cells) expressing vastly different amounts of EGF receptor on the cell surface. To our knowledge, none of the previous studies that examined EGF-stimulated tyrosine phosphorylation identified substrates exhibiting a bell-shaped response curve as a function of EGF concentration. As a comparison, we tested the phosphorylation of p120 Ras-GAP in response to EGF and found an increase in the tyrosine phosphorylation in a dose-dependent manner; similar results regarding p120 Ras-GAP have been reported earlier by others (36). Recent results by Ribon and Saltiel (25) differ from the results reported here; they found that stimulation of cells with a high concentration (100 ng/ml) of EGF results in tyrosine phosphorylation of Cas. The reason for this difference is unknown at present, but it may indicate cell type-specific differences in the response; Ribon and Saltiel (25) used PC-12 rat pheochromocytoma cells in their experiments.

Our results demonstrate that EGF increases Cas tyrosine phosphorylation at concentrations (0.5–5 ng/ml) significantly lower than the concentrations required to elicit a detectable increase in the tyrosine phosphorylation of several substrates of the EGF receptor, including p120 Ras-GAP (see Fig. 1; Refs. 35 and 36). In contrast, the concentrations that were found to stimulate a robust tyrosine phosphorylation of p120 Ras-GAP (>30 ng/ml) demonstrated a dramatic tyrosine dephosphorylation of Cas. These results suggest that EGF may trigger tyrosine phosphorylation of Cas and possibly other associated cellular components via a signal transduction pathway fundamentally distinct from that involving other known EGF receptor substrates. FAK, a focal adhesion protein whose tyrosine phosphorylation status closely parallels that of Cas, may exhibit a similar dose-response for EGF stimulation; studies by Rankin et al. have demonstrated that stimulation of Swiss 3T3 cells with low concentrations of EGF induces tyrosine phosphorylation of FAK (15). PDGF receptor may connect to similar intracellular signaling pathways as the EGF receptor; Casamassima and Rozengurt (14) have shown a similar bell-shaped dose-response curve for Cas tyrosine phosphorylation in response to PDGF stimulation. Low concentrations of EGF (present study) and PDGF (14) that stimulate tyrosine phosphorylation of focal adhesion proteins are mitogenic, raising the interesting possibility that focal adhesion proteins might play a role in amplifying and disseminating the mitogenic signal delivered by low, physiological concentrations of EGF and PDGF.

The intracellular signaling events leading to the modulation of the tyrosine phosphorylation status of Cas are not well characterized. Many of the stimuli leading to the enhancement of tyrosine phosphorylation of Cas have been shown to induce a rapid increase in stress fibers and in focal adhesions; these stimuli include integrin-mediated cell adhesion, as well as neurotropes, lysophosphatidic acid, and sphingosylphosphorylcholine (10, 44, 45). We found here that induction of Cas tyrosine phosphorylation by low concentrations of EGF requires the presence of intact cytoskeleton since the tyrosine phosphorylation can be prevented by cytochalasin D treatment. A similar situation has been observed when Cas phosphorylation has been induced by bombesin, low concentrations of PDGF, or integrin-mediated cell adhesion (14, 21, 22). It thus appears that tyrosine kinase(s) capable of phosphorylating Cas associate with focal adhesions upon EGF treatment and other stimuli, and an intact functional cytoskeleton may be required to bring together the various components of this signaling complex. One of the candidate kinases for phosphorylating Cas is FAK, which has been shown to directly interact with and mediate the tyrosine phosphorylation of Cas (26, 46) and which becomes activated by the same stimuli that induce tyrosine phosphorylation of Cas (47). Other candidates include Src family tyrosine kinases (26) and the FAK-homologue Pyk2/CAKβ/RAFTK/CADTK (48, 49), which also directly interact with Cas.

The effects on focal adhesions and stress fibers by extracellular stimuli are likely to be mediated by Rac and Rho, which belong to the Ras-related small G protein superfamily (see the Introduction and Refs. 3 and 5). Previous studies have demonstrated for the existence of a linear signal transduction pathway in the action of PDGF involving PI 3-kinase and Rac that leads to the tyrosine phosphorylation of Cas (14). Our studies show that stimulation of cells with low concentrations of EGF may initiate a similar intracellular signaling cascade, since the EGF-induced tyrosine phosphorylation of Cas was readily inhibitable by PI 3-kinase inhibitors. In contrast, neurotropes, lysophosphatidic acid, and sphingosylphosphorylcholine-induced tyrosine phosphorylation of Cas occurs in a PI 3-kinase
Cas plays a role in mitogenic signaling warrants further experimental work.

Not much is known about signaling pathways that induce tyrosine dephosphorylation of Cas. When cells are stimulated with insulin (18) or with high concentrations of EGF (present study), the dephosphorylation of focal adhesion proteins correlates with actin stress fiber breakdown and focal adhesion disassembly; the exact cause-effect relationship between the dephosphorylation events and focal adhesion disassembly is not known. It is possible that dephosphorylation of Cas takes place upon activation of tyrosine phosphatases in response to the extracellular stimuli. Candidate phosphatases include PTP1B and PTP-PEST, which have been shown to directly interact with and dephosphorylate Cas (50, 51). Interestingly, PTP-PEST was recently demonstrated to become recruited to activated EGF receptor complexes (52). In one study, insulin-induced tyrosine dephosphorylation of FAK and paxillin was found to be mediated by protein phosphatase SHP2 (Ref. 53; but see also Ref. 17); SHP2 is also a known component of the EGFR signaling pathway (38). Dephosphorylation may also take place through inactivation of tyrosine kinases; Tobe and co-workers (54) found that C-terminal Src kinase Csk is also involved in insulin’s regulation of the phosphorylation levels of the focal adhesion proteins, possibly through inactivation of the kinase activity of Src family kinases.

Recent reports have shown that integrin-dependent cell adhesion and stimulation of cells with neuropeptides, bioactive lipids, and low concentrations of PDGF can induce an SH2-mediated association of Crk with Cas (14, 26). Tyrosine-phosphorylated Cas also binds to Src and Nck in an SH2-dependent manner (27, 55). Our results demonstrate that low concentrations of EGF induce the formation of a Cas-Crk complex in intact RatER cells that is dependent on the integrity of the SH2 domain including SH2G (56, 57), which is a guanine nucleotide exchange factor for the small GTP-binding protein Rap-1 (58). Interestingly, recent work by Okayama and co-workers suggests that the oncogenic growth signal from EGFR receptor to Ras is predominantly mediated by Crk in rat fibroblasts (59). The interaction between Cas and Crk may be important in regulating the subcellular localization of Crk or the activity of the downstream effectors in EGF signal transduction pathways. The possibility that tyrosine phosphorylation of Cas plays a role in mitogenic signaling warrants further experimental work.

REFERENCES

1. Schlessinger, J., and Ullrich, A. (1992) Neuron 9, 383–391
2. Ridley, A. J., and Hall, A. (1992) Cell 70, 389–399
3. Ridley, A. J., Paterson, H. F., Johnston, C. L., Diekmann, D., and Hall, A. (1994) J. Biol. Chem. 269, 2157–2163
4. Wadsworth, R., Wilkins, R. R., and Leder, P. (1995) J. Biol. Chem. 270, 34343–34351
5. Hall, A. (1994) Bioessays 16, 365–375
6. Wennstrom, S., Siegbahn, A., Yokote, K., Arvidsson, A. K., Heldin, C. H., Mori, T., and Ullrich, A. (1992) J. Biol. Chem. 267, 2175–2180
7. Wennstrom, S., Hawkins, P., Malmstrom, M., and Wadstrom, T. (1996) Sci. STKE 190, pl1
8. Pidoux, A., Desbrow, J., and Heyworth, W. (1997) J. Biol. Chem. 272, 24343–24345
9. Rankin, S., Matzner, M., and Rosenquist, E. (1995) FEBS Lett. 354, 315–319
10. Polte, T. R., and Hanke, S. K. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 10978–10982
11. Hanks, S. K., and Polte, T. R. (1997) BioEssays 19, 137–145
12. Ashworth, A., van der Rest, M., and Harris, A. L. (1993) Endocrinology 132, 2257–2264
13. Ashworth, A., van der Rest, M., and Harris, A. L. (1993) Endocrinology 132, 2257–2264
14. Casamassima, A., and Rozengurt, E. (1997) J. Biol. Chem. 272, 9363–9370
15. Rankin, S., Hoshmand-Rad, R., Claesson-Welsh, L., and Rozengurt, E. (1996) J. Biol. Chem. 271, 7829–7834
16. Pillay, T. S., Sasaki, T., and Olefsky, J. M. (1995) J. Biol. Chem. 270, 991–994
17. Yamauchi, K., Milarski, K. L., Saito, R., and Pessin, J. E. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 664–668
18. Knight, J. B., Yamashita, R., and Pessin, J. E. (1995) J. Biol. Chem. 270, 10199–10203
19. Sakai, R., Iwamoto, A., Hirano, N., Ogawa, S., Tanaka, T., Mano, H., Yazaki, Y., and Hirai, H. (1994) EMBO J. 13, 3748–3756
20. Petch, L. A., Boekholt, S. J., Bainton, A., Parsons, J. T., and Burridge, K. (1995) J. Cell Sci. 108, 1371–1379
21. Ruoslahti, E. (1987) Cell 50, 225–227
22. Ridley, A. J., and Hall, A. (1992) Curr. Opin. Cell Biol. 4, 1101–1107
23. Ellis, C., Moran, M., McCormick, F., and Pawson, T. (1990) Nature 343, 377–381
24. Schwartz, M. A., Schaller, M. D., and Ginsberg, M. H. (1995) Annu. Rev. Cell Dev. Biol. 11, 459–509
25. Fantl, W. J., Johnson, D. E., and Williams, L. T. (1993) Annu. Rev. Biochem. 62, 453–481
26. Vlahos, C. J., Matter, W. F., Hui, K. Y., and Brown, R. F. (1994) J. Biol. Chem. 269, 5241–5248
27. Morian, M. F., Koch, C. A., Anderson, D., Ellis, C., England, L., Martin, G. S., and Pawson, T. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 8622–8626
28. Ruoslahti, E. (1994) Science 266, 1576–1578
29. Ishino, M., Ohba, T., Sasaki, H., and Sasaki, T. (1995) Oncogene 11, 2331–2338
30. Alenina, N., and Milosz, D. (1996) Genes Dev. 10, 1341–1355
31. Law, S. F., Etojyk, J., Wang, B., Mysliwiec, T., Kruh, G., and Golemies, E. A. (1990) Mol. Cell Biol. 16, 3327–3337
32. Minegishi, M., Tachibana, K., Sato, T., Iwata, S., Nijima, Y., and Morimoto, C. (1996) J. Exp. Med. 184, 1365–1375
33. Margolis, B., Rhein, S. G., Felder, S., Mervic, M., Lyall, R., Levitski, A., Ullrich, A., Zibeller, A., and Schlessinger, J. (1989) Cell 57, 1101–1107
34. Ellis, C., Moran, M., McCormick, F., and Pawson, T. (1990) Nature 343, 377–381
35. Tatum, T. D., and Ullrich, A. (1992) EMBO J. 11, 3377–3382
36. Ellis, C., Moran, M., McCormick, F., and Pawson, T. (1990) Nature 343, 377–381
37. Schwartz, M. A., Schaller, M. D., and Ginsberg, M. H. (1995) Annu. Rev. Cell Dev. Biol. 11, 459–509