We demonstrate here that growth hormone (GH) stimulates the activation of Rap1 and Rap2 in NIH-3T3 cells. Full activation of Rap1 and Rap2 by GH necessitated the combined activity of both JAK2 and c-Src kinases, although c-Src was predominantly required. GH-stimulated Rap1 and Rap2 activity was also demonstrated to be CrkII-C3G-dependent. GH stimulated the tyrosine phosphorylation of C3G, which again required the combined activity of JAK2 and c-Src. C3G tyrosine residue 504 was required for GH-stimulated Rap activation. Activated Rap1 inhibited GH-stimulated activation of RalA and subsequent GH-stimulated p44/42 MAP kinase activity and Elk-1-mediated transcription. In addition, we demonstrated that C3G-Rap1-mediated CrkII enhancement of GH-stimulated JNK/SAPK activity. We have therefore identified a linear JAK2-independent pathway switching GH-stimulated p44/42 MAP kinase and JNK/SAPK activities.

The Rap proteins (Rap1 and Rap2) belong to the Ras-like small GTPase superfamily, which consists of at least 13 members (1). In this family, Ras has been extensively studied and was originally regarded as a transforming oncogene since Ras genes have been found mutated in about 15% of all human tumors (2). It possesses an important role in cell growth and differentiation as it has been demonstrated to be required for activation of p44/42 MAP kinase activity by a number of growth factors including growth hormone (GH) (3–5). Rap1 is closely related to Ras and was initially identified as a suppressor of the K-ras transformed phenotype (6). Structurally, Rap1 shares striking similarity with Ras in the effector domain. It has been suggested that Rap1 antagonizes Ras activity by sequestration of its target, Raf-1, in the inactive form (7). In addition, several studies have demonstrated that both Rap1 and Ras can bind the same effectors, such as Raf-1 and RalGDS (8). Rap2 exhibits 60% identity to Rap1 and shares most of the effector proteins with Ras and Rap1 (9). The function of Rap2 is more limited compared with Rap1, although an antagonistic effect of Rap2 on Ras-mediated transcription has been reported (10).

One common mechanism utilized by small GTPases to regulate cellular function is to cycle between the inactive GDP-bound state and the active GTP-bound state. Guanine nucleotide exchange factors (GEFs) facilitate GDP dissociation and allow the more abundant GTP to rebind, while GTPase-activating proteins (GAPs) accelerate GTP hydrolysis to complete the cycle (1, 7). C3G is a Rap-specific GEF since it predominantly catalyzes the guanine nucleotide exchange reaction for Rap1 and Rap2 (10, 11). C3G was originally identified as a major protein bound to the SH3 domain of c-Crk (12). There are three proline-rich sequences that bind to the SH3 domain of c-Crk in the central region of C3G and one C-terminal CDC25 homology domain catalyzing the GEF reaction of C3G (13). The function of the C3G N terminus remains unknown but recent studies have reported the association of p130cas to this region (14). C3G is activated by c-Crk-mediated membrane recruitment. Two isoforms of c-Crk protein are generated by alternative mRNA splicing. The larger form is CrkII, containing one SH2 domain and two SH3 domains, while the smaller form is CrkI, which lacks the C-terminal SH3 domain and one negative regulatory tyrosine residue compared with CrkII. CrkII is more abundant than CrkI in normal cells; therefore, CrkII is the major adaptor for C3G (15). A number of growth factors and cytokines stimulate the recruitment of the Crk-C3G complex to the membrane where tyrosine kinases are located such that C3G tyrosine residue 504 is phosphorylated with a resultant increase in its GEF activity (16, 17).

GH is the primary regulator of postnatal somatic growth and metabolism (18, 19). It utilizes special groups of signaling molecules to regulate the transcription of specific genes required for the above processes. These signaling molecules include: 1) receptor-tyrosine kinases (EGFR receptor) (20) and non-receptor-tyrosine kinases (JAK2, Ref. 3; c-Src; c-Fyn, Ref. 21; and FAK, Ref. 22), although in the case of the EGFR receptor it may be used simply as an adaptor protein; 2) members of the MAP kinase family including p44/42 MAP kinase (23), p38 MAP kinase (24), and JNK/SAPK (21) and their respective downstream effectors; 3) members of the insulin receptor substrate (IRS) group including IRS-1, -2, and -3, which may act as docking proteins for further activation of signaling molecules including phosphatidylinositol 3-kinase (25); 4) small Ras-like GTPases (26); and 5) STAT family members including STATs 1, 3, 5a, and 5b (27, 28), which constitute one group of signaling molecules involved in transcriptional regulation by GH. Although JAK2 is postulated to be required for GH signal transduction, our group has recently identified a JAK2-dependent pathway regulating GH-stimulated p44/42 MAP kinase activity (29). GH stimulated the formation of GTP-bound RalA and subsequent phospholipase D activation, required for the acti-
viation of p44/42 MAP kinase by GH in a c-Src-dependent manner.

Here we have demonstrated that cellular stimulation with GH results in the activation of both Rap1 and Rap2 in NIH-3T3 cells. The activation of Rap by GH was achieved by the combined JAK2- and c-Src-dependent tyrosine phosphorylation of C3G. GH-stimulated Rap1 activation was utilized to negatively modulate GH-stimulated p44/42 MAP kinase activity and subsequent Elk-1-mediated transcription through inhibition of Rap1 and c-Src. Conversely, GH-stimulated C3G-dependent activation of Rap1-enhanced JNK/SAPK activity and subsequent c-Jun-mediated transcription in response to GH. Rap1 is therefore a GH effector molecule activated in a JAK2-independent manner.

EXPERIMENTAL PROCEDURES

Materials—Recombinant human growth hormone (hGH) was a generous gift of Novo Nordisk (Singapore). CrkII monoclonal antibody, Ras monoclonal antibody, RaIA monoclonal antibody, and PY20 monoclonal antibody were obtained from Transduction Laboratories (Lexington, KY). JAK2 polyclonal antibody, C3G polyclonal antibody, c-Src polyclonal antibody, serum receptor, Rap1 polyclonal antibody, Rap2 polyclonal antibody, and protein A/G plus agarose were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Secondary anti-IgG antibodies and the enhanced chemiluminescence (ECL) kit were purchased from Amersham Biosciences. The p44/42 MAP kinase assay kit and SAPK/JNK assay kit were purchased from New England Biolabs (Beverly, MA). The transfection reagent Effectene was purchased from Qiagen (Hilden, Germany). The complete protease inhibitor mixture tablets were purchased from Roche Diagnostics (Mannheim, Germany). All other reagents were obtained from Sigma Chemical.

pET15b-GST-RalGDS-RBD construct encoding the 97 amino acids spanning RBD of RalGDS, pGEX4T3-GST-RalGDS) construct for GST-RalGDS-RBD containing amino acids 397–518 of human RalGDS and pGEX2T-RBD construct for GST-RalRaf1-RBD containing amino acids 51–131 of Raf1 (8, 30, 31) were the generous gifts of Dr. Johannes L. Bos (Utrecht, Netherlands). The wild-type and dominant-negative Rap1A plasmids were kindly provided by Dr. Alfred Wittinghofer (Dortmund, Germany). The dominant-negative c-Src plasmid was obtained from Dr. Joan S. Brugge (Boston, MA). The dominant-negative JAK2 plasmid was a kind gift of Dr. Olli Silvennoinen (Tampere, Finland). The wild-type CrkII expression vector and the wild-type and mutant form of C3G type CrkII expression vector and the wild-type and mutant form of Ras were generously provided by Dr. Michiyuki Matsuda (Tokyo, Japan). The dominant-negative Ras plasmid was purchased from Upstate Biotechnology. The fusion trans-activator plasmid (pFA-Elk-1) consisting of the DNA binding domain of Gal4 residues 1–147) and the transactivation domain of Elk-1 were purchased from Stratagene (La Jolla, CA). All plasmids were prepared with the plasmid maxiprep kit from Qiagen.

Cell Culture and Treatment—NIH-3T3 cells were grown at 37 °C in 5% CO2 in Dulbecco’s modified Eagle’s medium containing 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM l-glutamine. Prior to treatment, cells were deprived for 20–24 h in medium containing 0.5% fetal bovine serum. Unless otherwise indicated, the concentration of hGH was 50 ng/ml. This concentration of GH is within the physiological range for circulating rodent GH (52).

Rap, Ral, and Ras Activation Assays—Serum-deprived cells were stimulated with hGH as indicated and then lysed on ice for 15 min in Ral buffer (50 mM Tris-HCl, pH 7.5, 200 mM NaCl, 2.5 mM MgCl2, 10% glycerol, 1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na3VO4, and 1 tablet complete protease inhibitor mixture per 50 ml). After lysis samples were centrifuged at 14,000 rpm for 15 min, and the supernatants were precipitated by addition of equal volume of A/G plus agarose chromatography. The agarose beads were removed by centrifugation, and then the protein concentrations of the resulting supernatants were determined. For each immunoprecipitation, 500–1000 μg of protein was incubated with 4–8 μg of corresponding antibody for 4 h or overnight at 4 °C. Immunocomplexes were collected by incubating with 40 μl of protein A/G plus agarose for 1 h or overnight. Immunoprecipitates were washed three times with IP buffer (10 mM Tris-HCl, pH 7.4, 1% Triton X-100) for 1 h each, followed by washes with 1 mM EDTA, pH 8.0, 1 mM EGTA, pH 8.0, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na3VO4, 1 tablet complete protease inhibitor mixture per 50 ml. The bound proteins were eluted in Laemmli sample buffer and then resolved by 8–10% SDS-PAGE.

Western Blot Analysis—After SDS-PAGE, proteins were transferred to nitrocellulose membranes. The membranes were blocked with 5% nonfat dry milk in phosphate-buffered saline with 0.1% Tween-20 (PBS-T) for 1 h at 22 °C. Blots were then immunolabeled with the desired antibodies for 1 h at 22 °C. For reblotting, membranes were reblocked and immunolabeled as described above.

p44/42 MAP Kinase Assay—p44/42 MAP kinase assays were performed according to the manufacturer’s instructions. Briefly, cells were lysed at 4 °C in lysis buffer provided, and the cell extract containing 200 μg of protein per sample was incubated for 4 h or overnight with 15 μl of immobilized phophospecific p44/42 MAP kinase (Tyr-202/Tyr-204) monoclonal antibody. The pellets were washed twice with 500 μl of lysis buffer and twice with 500 μl of kinase assay buffer provided. The kinase reactions were performed in the presence of 2 μg of Elk-1 fusion protein and 200 μM ATP at 30 °C for 30 min. Elk-1 phosphorylation was detected by use of a specific phospho-Elk1 (Ser-383) antibody.

SAPK/JNK Assay—SAPK/JNK assays were performed according to the manufacturer’s instructions. Briefly, cells were lysed at 4 °C in lysis buffer provided, and the cell extract containing 250 μg of total protein per sample was incubated overnight at 4 °C with 20 μl of c-Jun fusion protein beads. The pellets were washed twice with 500 μl of lysis buffer and twice with 500 μl of kinase assay buffer provided. The kinase assay was performed in the presence of 100 μl ATP at 30 °C for 30 min. c-Jun phosphorylation was detected by use of a specific phospho-c-Jun (Ser-63) antibody.

Elk-1 Reporter Assay—Cells were cultured to 60–80% confluence in 6-well plates and transfected with 0.4 μg of the reporter plasmid pFR-Luc, 8 ng of the fusion trans-activator plasmid pFA-Elk1, and 1 μg of the expression plasmid as indicated or empty vector in each well. 25 μl of Effectene was used for each microgram of DNA according to the manufacturer’s instructions. DNA-lipid complex was diluted in medium containing 2% fetal bovine serum for 16–20 h. 50 ng hGH was added for an additional 24 h. The cells were washed in cold phosphate-buffered saline twice and then lysed with 150 μl of 1X lysis buffer (25 mM Tris-phosphate, pH 7.8, 2 mM EDTA, 2 mM EGTA, 2 mM dithiothreitol, 10% glycerol, 1% Triton X-100) for 20 min, and supernatant was collected by centrifugation at 14,000 × g for 15 min. The luciferase activity was detected and normalized by protein content.

c-Jun Reporter Assay—Cells were cultured to 60–80% confluence in 96-well plates and transfected with 0.2 μg of the reporter plasmid pFR-Luc, 4 ng of the fusion trans-activator plasmid pFA-Elk1, and 1 μg of the expression plasmid as indicated or empty vector in each well. 25 μl of Effectene was used for each microgram of DNA according to the manufacturer’s instructions. DNA-lipid complex was diluted in medium containing 5% fetal bovine serum for 12 h. 50 ng hGH was added for an additional 18 h. The cells were washed in cold phosphate-buffered saline twice and then lysed with 150 μl of 1X lysis buffer (25 mM Tris-phosphate, pH 7.8, 2 mM EDTA, 2 mM EGTA, 2 mM dithiothreitol, 10% glycerol, 1% Triton X-100) for 20 min, and supernatant was collected by centrifugation at 14,000 × g for 15 min. The luciferase activity was detected and normalized by protein content.

Statistical Analysis and Presentation of Data—All experiments were performed at least three times. Numerical data were expressed as mean ± S.D. Data were analyzed using the two-tailed t test or analysis of variance.
GH Activates Rap

**RESULTS**

**GH Stimulation of NIH-3T3 Cells Increases the Level of GTP-bound Rap1 and Rap2**—We utilized the GST fused RaI-GDS-RBD (33) as a specific probe to determine Rap1 and Rap2 activity in lysates of NIH-3T3 cells stimulated by GH. The GST-fused Ral-GDS-RBD protein recognizes only the active GTP-bound form of Rap1 and Rap2 but not the inactive GDP-bound form of these molecules (33). We observed an increased level of GTP-bound Rap1 upon cellular stimulation with GH, first at 2 min after GH addition, sustained to 15 min, and followed by a decline from 30 to 60 min (Fig. 1A). GH stimulation of NIH-3T3 cells also resulted in the formation of GTP-bound Rap2 within 2 min. However, GH-stimulated formation of GTP-bound Rap2 was not sustained, as observed for Rap1, and the level of GTP-bound Rap2 decreased immediately after 5 min (Fig. 1C). GH stimulation of NIH-3T3 cells did not alter Rap1 or Rap2 protein levels over the examined time period of stimulation (Fig. 1B and D). The GH-stimulated formation of Rap1-GTP and Rap2-GTP was also dose-dependent with the enhancement of Rap1-GTP and Rap2-GTP levels first observed at 0.5 nM GH. The maximal stimulation of Rap1-GTP was from 5 to 50 nM GH (Fig. 1E) while that of Rap2-GTP was at 5 nM GH (Fig. 1G). Thus Rap1 and Rap2 are two signaling molecules utilized by GH to exert its effect on cellular function.

GH-stimulated activation of Rap1 and Rap2 is regulated by cell density. NIH-3T3 cells were cultured until 40, 70, or 100% confluence before treatment with 50 nM GH for 2 min. The GST-linked probe RaI-GDS-RBD, which recognizes only the GTP-bound form of Rap was used to separate Rap-GTP from Rap-GDP. GTP-bound Rap1 (A) and Rap2 (C) were visualized by Western blot analysis. Total cellular Rap1 (B) and Rap2 (D) were also determined in total cell lysates by Western blot analysis as protein loading control. The results presented are representative of a minimum of three independent experiments.

5 to 50 nM GH (Fig. 1E) while that of Rap2-GTP was at 5 nM GH (Fig. 1G). Thus Rap1 and Rap2 are two signaling molecules utilized by GH to exert its effect on cellular function.

GH-stimulated activation of Rap1 and Rap2 are cell density-dependent—During the course of experimentation, an effect of cell density in monolayer culture on the ability of GH to stimulate Rap1 and Rap2 activity was noticed. We therefore compared Rap activity under conditions of increasing cell density that approximated 40, 70, and 100% cell confluence, respectively. A decrease of both basal and GH-stimulated Rap1 and Rap2 activity was observed with increasing cell density. Thus, GH stimulation of NIH-3T3 cells at 100% confluence failed to stimulate the formation of Rap1-GTP and Rap1-GTP and Rap2-GTP was not due to decreased Rap protein as the total cellular level of both Rap1 and Rap2 was equivalent at different cell densities (Fig. 2, B and D). GH activation of Rap1 and Rap2 was therefore cell density-dependent.

**Full Activation of Rap1 and Rap2 by GH Requires both JAK2 and c-Src**—GH activates both JAK2 and c-Src kinases independent of the other (29). We have previously demonstrated that two other small Ras-like GTPases, RaIA and RaIB, require the activity of both c-Src and JAK2 to be fully activated by GH (29). We therefore next examined the requirement of JAK2 and c-Src for GH-stimulated activation of Rap1 and Rap2. Upon forced expression of the JAK2 kinase-deficient mutant (K882E) (34), both the basal and GH-stimulated formation of Rap1-GTP and Rap2-GTP were diminished, but GH stimulation of cells still resulted in increased Rap1 and Rap2 activity (Fig. 3, A and C). We have previously demonstrated the efficacy of forced expression of JAK2-K882E to prevent GH-stimulated activation of JAK2- and JAK2-dependent signal transduction (29). Forced expression of the c-Src kinase inactive mutant (K295R/Y527F) (35) also abrogated the ability of GH to stimulate the formation of GTP bound Rap1 and Rap2 (Fig. 3, A and C) to a greater extent than that observed with JAK2-K882E. Co-transfection of cDNA for both JAK2-K882E and c-Src-K295R/Y527F completely prevented the ability of GH to stimulate the forma-
and Rap2 (Fig. 4, A). GH-stimulated formation of GTP-bound Rap1 and Rap2 is increased by CrkII and C3G. Wild-type CrkII and C3G cDNAs were transiently transfected into NIH-3T3 cells before cell stimulation with 50 nM GH for 2 min. The GST-linked probe Ral-GDS-RBD, which recognizes only the active GTP-bound form of Rap1 and Rap2, was used to separate Rap-GTP from the inactive Rap-GDP. GTP-bound Rap1 (A) and Rap2 (C) were visualized by Western blot analysis. Total cellular Rap1 (B) and Rap2 (D) were also determined in total cell lysates by Western blot analysis as protein loading control. The forced expression of JAK2-K882E and c-Src-K295R/Y527F is indicated (E, F). The results presented are representative of a minimum of three independent experiments.

**C3G Tyrosine Phosphorylation Is Required for GH-stimulated Rap1 and Rap2 Activation—**Tyrosine phosphorylation of C3G has been reported to be required for the GEF activity necessary for Rap1 activation (17). We therefore first examined whether GH stimulation of NIH-3T3 cells resulted in tyrosine phosphorylation of C3G. As observed in Fig. 5A, GH indeed stimulated the tyrosine phosphorylation of C3G. GH-stimulated tyrosine phosphorylation of C3G was first observed at 1 min, persisted to 15 min, and then declined at 30–60 min after stimulation with GH. Equivalent loading of immunoprecipitated C3G was demonstrated by reprobing of the membrane for C3G (Fig. 5B).

To determine the kinases responsible for hGH-stimulated tyrosine phosphorylation of C3G we utilized the kinase-deficient mutants of both JAK2 (JAK2-K882E) and c-Src (c-Src-K295R/Y527F). Similar to the pattern observed with GH-stimulated formation of GTP-bound Rap1 and Rap2 (above), removal of the activities of both kinases was required for complete prevention of GH-stimulated C3G tyrosine phosphorylation (Fig. 5C). Equivalent loading of immunoprecipitated C3G was demonstrated by reprobing of the membrane for C3G (Fig. 5D). Forced expression of the kinase-deficient mutants of both JAK2 and c-Src was demonstrated by Western blot analysis (Fig. 5, E and F). Thus GH-stimulated tyrosine phosphorylation of C3G required the combined activities of both JAK2 and c-Src.

We next examined whether tyrosine phosphorylation of C3G was required for GH stimulated Rap1 and Rap2 activation. It has been reported that tyrosine 504 of C3G is the critical tyrosine residue required for guanine nucleotide exchange ac-
Rap1 inhibits GH-stimulated p44/42 MAP kinase activity and Elk-1-mediated transcription—Among the different Rap1 effectors, p44/42 MAP kinase has been extensively studied (1). Rap1 has been reported to either stimulate or inhibit p44/42 MAP kinase activity depending on the cellular context (1). p44/42 MAP kinase is also activated by GH (23) to exert pleiotropic cellular effects (19) and its mechanism of activation has been extensively studied (3, 26, 29). We therefore first examined the effect of forced expression of Rap1 on the ability of GH to stimulate p44/42 MAP kinase activity. GH stimulation of vector-transfected NIH-3T3 cells resulted in a rapid and prolonged activation of p44/42 MAP kinase activity such that at 60 min after GH stimulation, p44/42 MAP kinase activity was still higher than in the basal state (Fig. 6A). Forced expression of Rap1 did not affect the ability of GH to activate p44/42 MAP kinase but prevented the sustained activation of p44/42 MAP kinase (Fig. 6A). Thus, p44/42 MAP kinase activity was not detectable as early as 30 min after stimulation with GH in the presence of forcibly expressed Rap1. Concordantly, forced expression of dominant-negative Rap1S17N prolonged GH-stimulated p44/42 MAP kinase activity in comparison to vector-transfected control (Fig. 6D). Thus, markedly less diminution of p44/42 MAP kinase activity was observed at both 30 and 60 min after GH stimulation when Rap1 activation by GH was inhibited.

Activation of p44/42 MAP kinase by GH subsequently results in Elk-1-mediated transcription (36) and has been suggested to depend on MAP kinase activity, which is sustained after more than 30 min of cell stimulation (37). We therefore examined the effect of forced expression of both wild-type Rap1 and Rap1S17N on the ability of GH to stimulate Elk-1-mediated transcription. Forced expression of wild-type Rap1 completely prevented GH-stimulated Elk-1-mediated transcription that was observed in the vector-transfected control (Fig. 6G). Rap1S17N consistently enhanced the ability of GH to stimulate Elk-1-mediated transcription (Fig. 6G). Rap1 therefore negatively regulates the ability of GH to maintain sustained activation of p44/42 MAP kinase activity and subsequent Elk-1-mediated transcription.

Ras and Rap Are Activated by GH Independent of the Other—We have previously demonstrated that activation of two small GTPases by GH, RalA and RalB, is partially Ras-dependent (29). We therefore examined the effect of forced expression of GTPases by GH, RalA and RalB, is partially Ras-dependent (29). We therefore examined the effect of forced expression of both wild-type Rap1 and Rap1S17N on the ability of GH to stimulate Elk-1-mediated transcription. Forced expression of wild-type Rap1 completely prevented GH-stimulated Elk-1-mediated transcription that was observed in the vector-transfected control (Fig. 6G). Rap1S17N consistently enhanced the ability of GH to stimulate Elk-1-mediated transcription (Fig. 6G). Rap1 therefore negatively regulates the ability of GH to maintain sustained activation of p44/42 MAP kinase activity and subsequent Elk-1-mediated transcription.
However, as shown in Fig. 7, A and C, forced expression of either wild-type or dominant-negative mutant of Ras did not alter the ability of GH to stimulate the formation of GTP-bound Rap1 or Rap2. We next examined whether Rap affected the ability of GH to activate Ras. Forced expression of wild-type Rap1 did not alter the ability of GH to stimulate the formation of GTP-bound Ras (Fig. 7F). Concordantly, forced expression of Rap1S17N was without effect on GH-stimulated Ras activity (Fig. 7F). Forced expression of Ras and Rap1 proteins was demonstrated by Western blot analysis (Fig. 7, E and H).

**Rap1 Inhibits GH-stimulated Elk-1-mediated Transcription through Inactivation of RalA—**RalGDS, the Rap1 effector, is also a Ral specific GEF (38, 39) providing a potential mechanism for the regulation of Ral activity by Rap1 (40). We have previously demonstrated that GH stimulates the formation of GTP-bound RalA required for full p44/42 MAP kinase activation by GH and subsequent Elk-1-mediated transcription (29). We therefore examined whether Rap1 inhibition of GH-stimulated Elk-1-mediated transcription was via modulation of GH-stimulated RalA activity. GH stimulation of NIH-3T3 cells resulted in robust formation of GTP-bound RalA as previously reported by us (29). Forced expression of wild-type Rap1 dramatically inhibited the ability of GH to stimulate the formation of GTP-bound Rap1 (Fig. 8A). Concordantly, forced expression of the dominant-negative Rap1S17N enhanced the ability of GH to stimulate the formation of GTP-bound Rap1 (Fig. 8A). Forced expression of Rap1 and Rap1S17N was demonstrated by Western blot analysis (Fig. 8C) and did not affect total cellular levels of RalA (Fig. 8B). We next examined the interaction between Rap1 and RalA for the ability of GH to stimulate Elk-1-mediated transcription as an indicator of p44/42 MAP kinase activity. GH-stimulated Elk-1-mediated transcription was inhibited by forced expression of Rap1 and dramatically enhanced by forced expression of RalA (Fig. 8D). Forced expression of Rap1 concomitant with RalA abrogated the ability of RalA to enhance GH-stimulated Elk-1-mediated transcription. Rap1S17N slightly enhanced the fold stimulation by GH of Elk-1-mediated transcription. Dominant-negative RalA completely prevented GH-stimulated Elk-1-mediated transcription in cells transfected either with empty vector or with Rap1S17N (Fig. 8D). Thus, Rap1 influences the ability of GH to activate p44/42 MAP kinase activity and subsequent Elk-1-mediated transcription by modulation of GH-stimulated formation of GTP-bound RalA.

C3G and Rap1 Are Utilized by CrkII to Enhance GH-stimulated JNK/SAPK Activity and Subsequent c-Jun-mediated Transcription—We have previously reported that CrkII served as a molecular switch for the selective activation of JNK/SAPK by GH and concomitant inactivation of GH-stimulated p44/42 MAP kinase (41). We therefore examined whether C3G and Rap1 are required for CrkII enhancement of GH-stimulated JNK/SAPK activity. As demonstrated in Fig. 9A, forced expression vectors for wild-type Rap1 or Rap1S17N and stimulated with 50 nM GH for the indicated time periods, p44/42 MAP kinase activity was determined as described under “Experimental Procedures.” GH-stimulated p44/42 MAP kinase activity in the presence of transiently transfected wild-type Rap1 or Rap1S17N are presented in A and D, respectively. The level of the transfected Rap1 and Rap1S17N were shown as B and E. Densitometric evaluation of the effects of Rap1 and Rap1S17N on GH-stimulated p44/42 MAP kinase activation are presented in C and F. G, Rap1 inhibits GH-stimulated Elk-1-mediated transcription. NIH-3T3 cells were transiently transfected with the expression vectors for wild-type Rap1 or Rap1S17N together with pPR-Luc and pFA-Elk-1. The GH-stimulated Elk-1-mediated transcription was determined by measuring luciferase activity as described under “Experimental Procedures.” Data presented are mean ± S.E. of triplicate determinations. Experiments were repeated three times.
tion of either CrkII or C3G enhanced GH-stimulated JNK/SAPK activity. Forced co-expression of both CrkII and C3G further enhanced both basal and GH-stimulated JNK/SAPK activity (Fig. 9A). Expression of the inactive C3G-Y504F mutant did not affect GH-stimulated JNK/SAPK activity in NIH-3T3 cells. It could, however, prevent the enhanced GH-stimulated JNK/SAPK activity observed upon forced expression of CrkII (Fig. 9A). Forced expression of CrkII, C3G, and C3G-Y504F was demonstrated by Western blot analysis (Fig. 9, B and C). We next examined the effect of forced expression of CrkII and C3G on the ability of GH to stimulate c-Jun-mediated transcription. GH stimulation of NIH-3T3 cells resulted in minimal stimulation of c-Jun-mediated transcription (Fig. 9D). Forced expression of either CrkII or C3G enhanced the ability of GH to stimulate c-Jun-mediated transcription (Fig. 9D). Concordant with JNK/SAPK activity, forced co-expression of both CrkII and C3G enhanced both basal and GH-stimulated c-Jun-mediated transcription. Again, similar to the effect observed with JNK/SAPK activity, the C3G-Y504F mutant did not affect GH-stimulated c-Jun-mediated transcription per se but did prevent the enhanced GH-stimulated c-Jun-mediated
GH Activates Rap

Fig. 9. CrkII-dependent hGH-stimulated JNK/SAPK activation and subsequent c-Jun-mediated transcription is via C3G and Rap1. A–E and F–G, NIH-3T3 cells were transiently transfected with the expression vectors for wild-type CrkII in the presence or absence of wild-type C3G, tyrosine site mutant C3G-Y504F, wild-type Rap1, or dominant-negative Rap1S17N, and stimulated with 50 nM GH for the indicated time periods. JNK/SAPK kinase activity was determined as described under "Experimental Procedures." The phosphorylation level of c-Jun fusion protein indicative of JNK/SAPK is shown in A and E. The level of expressed CrkII, C3G and Rap1 are shown in B, F, C, and G, respectively. The results presented are the representative of at least three independent experiments. D and H, C3G and Rap1 mediated CrkII-enhanced GH-stimulated c-Jun-mediated transcription. NIH-3T3 cells were transiently transfected with the expression vector for transcription observed upon forced expression of CrkII (Fig. 9D).

We next examined whether Rap1 is required for CrkII enhancement of GH-stimulated JNK/SAPK activity. As demonstrated in Fig. 9E, forced expression of either CrkII or Rap1 enhanced GH-stimulated JNK/SAPK activity. Forced co-expression of both CrkII and Rap1 dramatically enhanced both basal and GH-stimulated JNK/SAPK activity (Fig. 9E). Expression of the dominant-negative Rap1S17N did not affect GH-stimulated JNK/SAPK activity per se. It could, however, largely prevent the enhanced GH-stimulated JNK/SAPK activity observed upon forced expression of CrkII (Fig. 9E). Forced expression of CrkII, Rap1, and Rap1S17N was demonstrated by Western blot analysis (Fig. 9, F and G). We next examined the effect of forced expression of CrkII and Rap1 on the ability of GH to stimulate c-Jun-mediated transcription. GH stimulation of NIH-3T3 cells resulted in minimal stimulation of c-Jun-mediated transcription (Fig. 9H). Forced expression of either CrkII or Rap1 enhanced the ability of GH to stimulate c-Jun-mediated transcription. Forced co-expression of both CrkII and Rap1 resulted in a dramatic enhancement of GH-stimulated c-Jun-mediated transcription (Fig. 9I). Similar to the effect observed with JNK/SAPK activity, the dominant-negative Rap1S17N did not affect GH-stimulated c-Jun-mediated transcription per se but did prevent the enhanced GH-stimulated c-Jun-mediated transcription observed upon forced expression of CrkII (Fig. 9H).

DISCUSSION

In the present study we have demonstrated that cellular stimulation with GH results in a rapid activation of the Ras-like small GTPases, Rap1 and Rap2. To date, Ras and Raf, the other two close relatives of Rap, together with Rac have been reported to participate in GH signal transduction (3, 29, 42), suggestive of a significant role for small Ras-like GTPases in the cellular effects of GH. Rap1 has also been demonstrated to be activated by other members of the cytokine receptor superfamily to which the GH receptor belongs, including EPO and IL-3 (43).

It is interesting to note that cell density exerted a significant inhibitory effect on both basal and GH-stimulated Rap activity. This is consistent with a previous report demonstrating that basal Rap1 activity is cell density-dependent (44). Rap1 has also been demonstrated to be activated upon cell adhesion to ECM and is implicated in integrin-mediated cell adhesion in various cells in response to diverse extracellular stimuli (1). Integrins, the transmembrane glycoproteins that usually bind cells to ECM, may also bind cells to cells in a calcium-dependent manner (45). The involved integrins are heterodimers composed of both the α-subunit and the β-sub unit. Although integrins may participate in intercellular interaction, most cell-cell adhesions are mediated by cadherin that is linked with the actin cytoskeleton through β-catenin (46). Recently, a novel β-catenin-interacting protein with a putative Rap1GEF activity has been identified (47), suggesting a role for Rap1 in the regulation of cell-cell contact. Furthermore, the yeast Rap1 homologue, Bud1, can directly activate Cdc24, an exchange factor for Cdc42, which is involved in the recruitment of actin cytoskeleton to the bud site (48). Rap2 has been demonstrated

CrkII or in the presence of the expression vectors for C3G, tyrosine site mutant C3G-Y504F, Rap1, or dominant-negative form of Rap1 (Rap1S17N) together with pFR-Luc and pFA-c-Jun. GH-stimulated c-Jun-mediated transcription was determined by measuring luciferase activity as described under "Experimental Procedures." Data presented are mean ± S.E. of triplicate determinations. Experiments were repeated three times.
to bind specifically with actin filaments to interact with cytoskeletal components (49). We have also observed a GH-dependent association between Rap1 and actin by co-immunoprecipitation. Proliferation of NIH-3T3 cells is known to be highly sensitive to contact inhibition (50). In this regard it is interesting that we have observed that autocrine production of GH in human mammary carcinoma cells results in disassembly of adherens junctions and loss of intercellular contact. How this phenomenon relates to the inability of GH to activate Rap1 in confluent cells remains to be determined.

We have demonstrated here that full activation of Rap1 and Rap2 by GH requires the combined activity of both JAK2 and c-Src, although c-Src is the predominant kinase utilized by GH for this purpose. We have therefore described another JAK2-independent mechanism by which GH affects cellular function. In addition, our findings have determined that JAK2 and c-Src activate Rap through tyrosine phosphorylation and activation of C3G, a Rap-specific GEF. These results are concordant with our recent observation that GH-stimulated formation of both GTP-bound RalA and RalB also required both c-Src and JAK2 (29). We have previously demonstrated that GH activates JAK2 and c-Src independent of, and parallel to, each other (29). The two kinases obviously converge for joint phosphorylation of C3G required for Rap activation by GH and the relative contribution of each kinase may simply depend on the relative expression of JAK2 or c-Src in a particular cell type. Both JAK and c-Src have previously been demonstrated to be utilized for activation of Rap1 (51, 52). One example of JAK-dependent activation of Rap1 is the requirement of JAK1 and Tyk2 for Rap1 activation in type I IFN signaling (52). Src-dependent Rap1 activation is essential for integrin-mediated cell adhesion and formation of focal adhesion structures (53). The adaptor protein CrkII has been identified to mediate Src-dependent Rap1 activation (54). We have previously demonstrated that CrkII is constitutively associated with C3G (21), and GH-stimulated Rap activation is CrkII-C3G-dependent (this study). CrkII possesses a pivotal role in GH signal transduction (41) and is central to the formation of a large multiprotein signaling complex upon GH stimulation of cells (21). Thus, CrkII may recruit C3G to the vicinity of JAK2 to facilitate C3G tyrosine phosphorylation by JAK2. FAK may act as a bridge between CrkII and JAK2 since GH can stimulate the association of FAK with both JAK2 and CrkII (21, 22). c-Src activated by GH also forms part of the multi-protein complex centered around CrkII (21). Interestingly, an increased association stimulated by GH is also observed between FAK and c-Src (21) and therefore CrkII may facilitate the formation of this triple kinase complex together with C3G. In any case, cellular stimulation with GH results in the tyrosine phosphorylation of C3G. It has been reported that the phosphorylation of tyrosine residue 504 (Tyr-504) in C3G is critical for C3G-dependent Rap1 activation, presumably as phosphorylation of Tyr-504 in C3G represses the negative regulation of C3G activity by its N-terminal domain (17). Consistent with this observation, the C3G-Y504F mutant, in which Tyr-504 is replaced by the nonphosphorylatable residue phenylalanine, prevented GH-stimulated formation of GTP-bound Rap. Both JAK2 and c-Src must therefore phosphorylate this same residue to achieve activation of Rap1 by GH. CrkII-C3G-dependent activation of Rap1 therefore constitutes another JAK2-independent pathway utilized by GH.

We have demonstrated here that the forced expression of wild-type Rap1 prevented the prolonged activation of p44/42 MAP kinase activity observed after cellular stimulation with GH. Concordantly, forced expression of the dominant-negative mutant of Rap1 prolonged the activation of p44/42 MAP kinase by GH. Several studies have previously demonstrated that Rap1, or mutants thereof, can inhibit the p44/42 MAP kinase pathway (1). For example, a constitutively active mutant of Rap1 was reported to inhibit LPA or EGF induced p44/42 MAP kinase activity and Ras-p44/42 MAP kinase stimulated IL-2 expression (55-57). IL-1-stimulated activation of Rap1 was also observed to repress Ras-mediated activation of p44/42 MAP kinase signaling (43). These observations support a model of Rap function stating that Rap1 is a functional antagonist of Ras activity; originating from the demonstration of Rap1 repression of the K-ras transformed phenotype in NIH-3T3 cells (5). There are therefore two possible mechanisms for Rap1 to inhibit Ras signaling. First, Ras and Rap1 may possess a regulator and effector relationship in the same pathway. However, it has been demonstrated that Rap1 is not upstream of Ras (57), which is also observed in this study and here we report that GH-stimulated Rap activation is not Ras-dependent. Therefore, a more plausible mechanism is that Ras and Rap1 are involved in distinct pathways while competing for the same effector(s). Due to the striking structural similarity in the effector domain of Rap1 and Ras (58), it has been proposed that Rap1 interferes with Ras signaling pathway by sequestering the Ras substrate Raf-1 kinase. However, although Rap1 binds to Raf-1 in vitro and in vivo (8, 55, 59), there is still no demonstration to date that Rap1 inhibits Raf-1 kinase activity (1). Furthermore, Raf kinase-independent regulation of p44/42 MAP kinase by Rap1 has been identified recently (44). GH-stimulated p44/42 MAP kinase activation has been demonstrated to require both Ras and Raf-1 activity (3). However, we observed no association between Rap1 and Raf-1 in NIH-3T3 cells either in the quiescent or GH-stimulated state. We have, however, demonstrated here that Rap1 inhibits GH-stimulated formation of GTP-bound RalA. We have previously reported that forced expression of RalA prolongs GH-stimulated p44/42 MAP kinase activity (29). The inhibition of the GH-stimulated formation of GTP-bound RalA by Rap1 is presumably mediated by RalGDS, a putative effector shared by Ras and Rap1. As a member of the RalGEF family, RalGDS contains RBDs that bind to activated Ras or Rap1 in vitro and in vivo (38). Ras-dependent Raf activation has been demonstrated to be inhibited by Rap1 due to the retention of RalGDS to the compartment where Rap1 is located, instead of being recruited by Ras to the site of Raf (40). Subcellular localization of Rap1 is mainly at cytosol and the perinuclear compartment, different to that of Ras and Rap1 localized at the plasma membrane. RalGDS is found in the cytosol and can be recruited to plasma membrane by Ras in order to activate Raf (40). It has been reported for some time that co-localization of Ras and Rap1 on the plasma membrane is necessary for Raf activation in COS cells (60). Furthermore the localization of RalGDS to the plasma membrane is sufficient for Raf activation (40). Both Ras and Rap1 have the binding domain specific for RalGDS, however, Rap1 has higher affinity to RalGDS than Ras and promotes the translocation of RalGDS to the compartment where Raf is not found, providing a mechanism that Rap1 sequesters RalGDS to prevent Raf activation (61).

We have previously reported that GH-stimulated formation of GTP-bound RalA and RalB occurs in a biphasic manner (29). It is therefore interesting to note that GH-stimulated activation of RalA occurs earlier than that of Rap1 and the trough of GH-stimulated RalA activity is coincident with the sustained phase of GH-stimulated Rap1 activation. Furthermore, when GH-stimulated formation of GTP-bound Rap1 decreased at 30 min, GH-stimulated RalA activity peaked simultaneously for

---

2 L. Ling and P. Lobie, unpublished observations.
3 S. Mukhina, H. Mertani, K. Guo, and P. E. Lobie, manuscript in preparation.
the second time. Rap1 is therefore presumably involved in a cellular mechanism to limit the ability of GH to maintain elevated p44/42 MAP kinase activity but without interference in the initial activation of p44/42 MAP kinase by GH. It is also noteworthy that GH can activate RalA even at a concentration as low as 0.005 nM whereas full activation of Rap1 by GH is observed at concentrations of 5–50 nM. Secretion of GH is sexually dimorphic in most species to date (62) and is responsible for male specific growth patterns. The sexually dimorphic pattern of secretion is characterized in males by consecutive peaks and troughs in GH serum concentration (62, 63). In rats, the peak values of GH can be greater than 200 ng/ml (about 8 nM) and trough values are less than 1 ng/ml (about 0.05 nM) (63). Our results suggest that Rap1, unlike RalA, would be activated by GH only when the pulsatile GH secretion reaches the peak which would subsequently attenuate RalA activity and subsequent p44/42 MAP kinase activity. How the differential activation of RalA and Rap1 relates to the sexually dimorphic response of mammals to GH needs to be determined.

p44/42 MAP kinase activity is also pertinent to aberrant signaling in human cancer and constitutive activation of this kinase has been observed in some tumors (64). Attenuation of GH-stimulated p44/42 MAP kinase activity by Rap1 would therefore limit the oncogenic potential of GH. The limiting effect of Rap1 on GH-stimulated p44/42 MAP kinase activity is consistent with previous reports concerning the ability of Rap1 to reverse oncogenic transformation (6). In agreement with our findings, other groups have also demonstrated that both LPA and EGF can induce a substantial Rap1 activation and Rap1V12 (constitutive Rap1-GTP) attenuates the activation of p44/42 MAP kinases by those mitogens (44, 57, 65). Furthermore, CrkII, identified in this report as an upstream activator of Rap1, has also been demonstrated previously to inhibit p44/42 MAP kinase activation by GH (41). Therefore we have identified a pathway mediated through CrkII-C3G-Rap1, which modulates GH-stimulated p44/42 MAP kinase activity by suppression of RalA. This negative regulatory pathway may be pivotal to ensure precise regulation of GH-stimulated p44/42 MAP kinase signaling.

We have previously demonstrated that CrkII is utilized by GH for activation of JNK/SAPK (21). Here we have further demonstrated that C3G-dependent activation of Rap1 is required for CrkII enhancement of GH-stimulated JNK/SAPK activation. C3G has previously been reported to be upstream of JNK/SAPK and a CrkII-C3G complex is believed to activate JNK/SAPK through a pathway involving the MLK family proteins (66, 67). However, neither the dominant-negative Rap1S17N nor functionally deficient C3G-Y504F can prevent hGH-stimulated JNK/SAPK activation in NIH-3T3 cells suggesting that there must also exist CrkII-C3G-independent pathways for the activation of JNK/SAPK by GH (see Fig. 10). One possible molecule is via the adaptor protein Nck, and we have previously demonstrated that Nck is phosphorylated by cellular stimulation with GH (21). Nck connects to the JNK/ SAPK pathway by association with SH3 domain-associated protein serine/threonine kinases such as Pak or NIK (68, 69). One recent report has also demonstrated that gastrin-stimulated JNK/SAPK activation is Src-dependent but CrkII-independent (70). It has been proposed multidomain scaffold proteins, such as JIP, axin, and arrestin regulate JNK activation in response to different stimuli (70). A SH2-dependent JNK/ SAPK activation by insulin has also been identified (71). This pathway is mediated by H-Ras and not CrkII, because Rac, known as the major downstream effector for CrkII-dependent JNK/SAPK activation, is not required for insulin-stimulated JNK/SAPK activation (71). Thus, GH may utilize the CrkII-independent pathways described above for the activation of JNK/SAPK, in addition to CrkII-C3G-Rap1 pathway described herein, in cells where the endogenous level of CrkII is minimal such as NIH-3T3 cells utilized for this study. CrkII may also utilize other effector molecules to activate JNK/SAPK in response to GH, such as Rac and R-Ras, which are required for v-Crk-dependent JNK/SAPK activation (72). However as the CrkII enhancement of GH-stimulated JNK/SAPK activity is largely inhibited by C3G-Y504F or Rap1S17N (this study), it is likely that C3G-Rap1 is the major pathway downstream of CrkII required for activation of JNK/SAPK by GH. The activation of JNK/SAPK by GH provides another pathway by which GH may affect cellular function. JNK/SAPK is involved in many cellular processes, including transcriptional regulation, proliferation and apoptosis (73) and it is likely that GH utilizes JNK/SAPK for some of these purposes. Although there is considerable evidence demonstrating that activation of JNK/SAPK by c-Jun can trigger apoptosis, reports have also accumulated that JNK/SAPK signaling to c-Jun can inhibit apoptosis and promote proliferation dependent on cell type and stimulus (74). In fibroblasts, the replacement of Ser-63 and Ser-73 of c-Jun by nonphosphorylatable alanines results in defective proliferation and loss of protection from apoptosis induced by UV irradiation (75). The phosphorylation of c-Jun on Ser-63 and Ser-73 by JNK/SAPK increases its transcriptional activity (76, 77). Thus, GH may utilize JNK/SAPK to execute its documented proliferative and anti-apoptotic effects (19) in a CrkII-dependent or -independent manner, determined by the expression level of CrkII in a specific cell line.
In summary, we have demonstrated here that GH stimulates the formation of GTP-bound Rap1 and Rap2 in NIH-3T3 cells. GH-stimulated activation of Rap is predominantly mediated by c-Src-dependent tyrosine phosphorylation of C3G. GH utilizes the inhibitory effect of Rap1 to limit activation of p44/42 MAP kinase pathway via inhibition of RaLα. In addition, we have demonstrated that the CrkII-C3G-Rap1 pathway is utilized by GH as a molecular switch from p44/42 MAP kinase signaling to JNK/SAPK signaling. A disintegrinizing GH utilization of the Ras-like small GTPases to regulate MAP kinase pathways is provided in Fig. 10. The identification of another JAK2-independent signaling pathway by GH will dramatically increase our understanding of the mechanisms utilized by GH to achieve its pleiotropic cellular effects.

REFERENCES

1. Bos, J. L., de Rooy, J., and Reedquist, K. A. (2001) Nat. Rev. Mol. Cell. Biol. 2, 369–377
2. Bos, J. L. (1989) Cancer Res. 49, 4682–4689
3. Winston, L. A., and Hunter, T. (1995) J. Biol. Chem. 270, 30837–30840
4. Burgering, B. M., and Bos, J. L. (1995) Trends Biochem. Sci. 20, 18–22
5. Marshall, C. J. (1996) Curr. Opin. Cell Biol. 8, 197–204
6. Kitayama, H., Sugimoto, Y., Matsuoka, T., Ikawa, Y., and Noda, M. (1989) Cell 56, 77–84
7. Bos, J. L. (1998) EMBO J. 17, 6767–6782
8. Okada, S., Matsuoka, M., Anafi, M., Pawson, T., and Pessin, J. E. (1998) EMBO J. 17, 2584–2595
9. Nanci, V., Woltiuis, R. M., de Tand, M. F., Janoueix-Lerosey, I., Bos, J. L., and de Gunzburg, J. J. (1999) J. Biol. Chem. 274, 8737–8745
10. Ohba, Y., Mochizuki, N., Matsu, K., Yamashita, S., Nakaya, M., Hashimoto, Y., Hamaguchi, M., Kurata, T., Nagashima, K., and Matsuoka, M. (2000) Mol. Cell. Biol. 20, 6074–6083
11. Gotoh, T., Hattori, S., Nakamura, S., Kitayama, H., Noda, M., Takai, Y., Kasahara, K., Matsu, H., Natsu, S., Takahashi, H., Kurata, T., and Matsuoka, M. (1995) Mol. Cell. Biol. 15, 6746–6753
12. Zhai, B., Huo, H., and Liao, K. (2001) Biochem. Biophys. Res. Commun. 286, 61–66
13. Matsuoka, M., Ota, S., Taninuma, R., Nakamura, H., Matsuoka, T., Takenawa, T., Nagashima, K., and Kurata, T. (1996) J. Biol. Chem. 271, 14468–14472
14. Kirsch, K. H., Georgescu, M. M., and Hanafusa, H. (1998) J. Biol. Chem. 273, 25673–25679
15. Matsuoka, T., Manaka, T., Nagata, S., Kojima, A., Kurata, T., and Shibuya, M. (1992) Mol. Cell. Biol. 12, 3482–3492
16. Kiyokawa, E., Mochizuki, N., Kurata, T., and Matsuoka, M. (1997) Crit. Rev. Oncog. 8, 329–342
17. Ichiba, T., Hashimoto, Y., Nakaya, M., Kurasaki, Y., Tanaka, S., Kurata, T., Mochizuki, N., and Matsuoka, M. (1999) J. Biol. Chem. 274, 14376–14381
18. Carter-Su, C., and Smit, L. S. (1996) Proc. Natl. Acad. Sci. 93, 81–82: discussion 82–83
19. Zhu, T., Goh, E. L., Graichen, R., Ling, L., and Lobie, P. E. (2001) Cell Signal. 13, 59–59
20. Yamauchi, T., Ueki, K., Tobe, K., Tamemoto, H., Sekine, N., Wada, M., Honjo, M., Matsuda, M. (1995) J. Biol. Chem. 270, 27544–27551
21. Pulverer, B. J., Kyriakis, J. M., Avruch, J., Nikolakaki, E., and Woodgett, J. R. (2000) J. Biol. Chem. 275, 7818–7825
22. Cook, S. J., Ruhfeldt, B., Albert, I., and McCormick, F. (1993) EMBO J. 12, 3475–3485
23. Pizon, V., Chardin, P., Lerosey, I., Olofsson, B., and Tavitian, A. (1988) Oncogene 3, 201–204
24. Herrmann, C., Horn, G., Spaargaren, M., and Wittinghofer, A. (1996) J. Biol. Chem. 271, 6797–6800
25. Kishida, S., Koyama, S., Matsuoka, K., Kishida, M., Matsuoka, Y., and Kikuchi, A. (1997) Oncogene 15, 2899–2907
26. Linnemann, T., Kiel, C., Herter, P., and Herrmann, C. (2002) J. Biol. Chem. 277, 7851–7857
27. Eden, S. (1979) Endocrinology 105, 555–560
28. Tannenbaum, G. S., and Martin, J. B. (1976) Endocrinology 98, 562–570
29. Seboli-Leopold, J. S. (2000) Oncogene 19, 6594–6599
30. Zwartkruis, F. J., Woltiuis, R. M., Nabben, N. M., Franke, B., and Bos, L. J. (1998) EMBO J. 17, 5956–5962
31. Tanaka, S., Ouchi, T., and Hanafusa, H. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 2556–2561
32. Tanaka, S., and Hanafusa, H. (1998) J. Biol. Chem. 273, 1281–1284
33. Bokoch, G. M., Wang, Y., Bohl, B. P., Sells, M. A., Quilliam, L. A., and Knaus, U. G. (1996) J. Biol. Chem. 271, 25746–25749
34. Su, Y. C., Han, J., Xu, S., Cobb, M., and Skolnik, E. Y. (1997) EMBO J. 16, 1279–1290
35. Dehez, S., Bierkamp, C., Kowalski-Chauvel, A., Daulhac, L., Escreit, C., Susini, C., Pradayrol, L., Fourmy, D., and Seva, C. (2002) Cell Growth Differ. 13, 375–385
36. Fukunaga, K., Naguchi, T., Takeda, H., Matozaki, T., Hayashi, Y., Itoh, H., and Kasuga, M. (2000) J. Biol. Chem. 275, 5298–5313
37. Mochizuki, N., Ohba, Y., Kobayashi, S., Otsuka, N., Graybeal, A. M., Tanaka, S., and Matsuoka, M. (2000) J. Biol. Chem. 275, 12667–12671
38. Herdegen, T., Skene, P., and Bahr, M. (1997) Trends Neurosci. 20, 227–231
39. Leppa, S., and Bohnmann, D. (1998) Oncogene 18, 6158–6162
40. Wisdom, J., Johnson, R. S., and Moore, C. (1999) EMBO J. 18, 188–197
41. Pulverer, B. J., Kyriakis, J. M., Avruch, J., Nikolakakis, E., and Woodgett, J. R. (1991) Nature 353, 670–674
42. Simeone, T., Binetruy, B., Mercier, D. A., Birrer, M., and Karin, M. (1991) Nature 354, 484–496
Src-CrkII-C3G-dependent Activation of Rap1 Switches Growth
Hormone-stimulated p44/42 MAP Kinase and JNK/SAPK Activities
Ling Ling, Tao Zhu and Peter E. Lobie

J. Biol. Chem. 2003, 278:27301-27311.
doi: 10.1074/jbc.M302516200 originally published online May 6, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M302516200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 77 references, 45 of which can be accessed free at
http://www.jbc.org/content/278/29/27301.full.html#ref-list-1