IQGAP1 binds several proteins including actin, calmodulin, E-cadherin, β-catenin, Cdc42, Rac1, and CLIP-170. The interaction with these targets enables IQGAP1 to participate in many cellular functions varying from regulation of the cytoskeleton to gene transcription. Here we show that extracellular signal-regulated kinase (ERK) 2 binds to IQGAP1. In vitro analysis with purified proteins demonstrated a direct interaction between ERK2 and IQGAP1. Moreover, binding occurred in cells as endogenous ERK2 co-immunoprecipitated with IQGAP1 from human breast epithelial cell lysates. The association between ERK2 and IQGAP1 was independent of epidermal growth factor. The in vivo interaction has functional significance. Manipulation of intracellular IQGAP1 levels significantly reduced growth factor-stimulated ERK1 and ERK2 activity. Similarly, stimulation of ERK1 and ERK2 activity by insulin-like growth factor I was reduced when IQGAP1 levels were changed. In contrast, overexpression of an IQGAP1 construct lacking the ERK2 binding region did not interfere with activation of ERK1 and ERK2 by epidermal growth factor. Our data disclose a previously unidentified communication between IQGAP1 and the ERK pathway and imply that IQGAP1 modulates the Ras/mitogen-activated protein kinase signaling cascade.

Extracellular signals are converted into cellular responses via a complex network of intersecting signaling pathways (1). A prominent component of intracellular signaling is the family of mitogen-activated protein (MAP)1 kinases. The archetypal MAP kinase pathway consists of a three-kinase cascade, a MAP kinase pathway, which in turn elicits a phosphorylation-dependent increase in the activity of the MAP kinase (2, 3). The MAP kinase then induces phosphorylation of a variety of cytosolic or nuclear targets, transcription factors, transcriptional adaptor proteins, and other protein kinases. Of the various mammalian MAP kinase pathways, the Ras/Raf/MEK/ERK cascade is the most studied. This cascade, triggered by a diverse range of stimuli acting through cell surface receptors, is under the control of the small G protein Ras (4). When GTP-bound, Ras binds to Raf and recruits it to the cell membrane where Raf is activated (5–7). Raf phosphorylates and activates the dual specificity protein kinases MEK1 and MEK2, which in turn stimulate ERK1 and ERK2 by catalyzing their phosphorylation (3). The Ras/Raf/MEK/ERK cascade is conserved in all eukaryotes, and ERK plays a vital role in several biological processes, particularly those involving cellular proliferation, differentiation, survival, and apoptosis (8).

IQGAP1 is a scaffolding protein with multiple protein-interacting domains (for reviews, see Refs. 9–11). These motifs include a calponin homology domain (CHD), four IQ motifs, and a RasGAP-related domain. Targets for IQGAP1 include calmodulin (12–14), Cdc42 (12, 13), Rac1 (13), actin (14, 15), β-catenin (16, 17), E-cadherin (16, 18), S100B (19) and CLIP-170 (20). Through interactions with these proteins, IQGAP1 participates in multiple fundamental cellular activities, including transcription, cell-cell attachment, and regulation of the cytoskeleton (9). The observation by Morgan’s group that ERK interacts with the CHDs of calponin and α-actinin (21) raised the possibility that IQGAP1 may bind ERK. Here, we present evidence that IQGAP1 and ERK2 associate both in vitro and in intact cells. This interaction has functional significance. The ability of epidermal growth factor (EGF) to stimulate phosphorylation of ERK1 and ERK2 was eliminated in cells in which endogenous IQGAP1 was specifically knocked down by siRNA. These data suggest that IQGAP1 contributes to the regulation of EGF-stimulated ERK activity.

**Experimental Procedures**

**Materials—**Tissue culture reagents were obtained from Invitrogen. Fetal bovine serum was purchased from BioWhittaker. Anti-ERK2 and anti-phospho-ERK antibodies were obtained from Pharmingen and Promega, respectively. Anti-MEK antibodies were obtained from Cell Signaling Technology. Anti-FLAG M2 and anti-Myc monoclonal antibodies (9E10.2) were manufactured by Santa Cruz Biotechnology and Transduction Laboratories, respectively. Anti-Myc monoclonal antibodies (9E10.2) were manufactured by Maine Biotechnology. The anti-IQGAP1 polyclonal antibody has been previously characterized (14). Secondary antibodies for enhanced chemiluminescence detection were from Amersham Biosciences. The MAP kinase assay kit was purchased from Cell Signaling Technology. All other reagents were of standard analytical grade.

**Cell Lines—**MCF-7 human breast epithelial cells, which stably over-express either pcDNA3 (termed MCF/V) or pcDNA3-myc-IQGAP1 (termed MCF/I cells), have been described previously (17, 22). MCF/I cells have 3-fold more IQGAP1 than MCF/V cells (22).

**Cell Culture and Transfection—**MCF-7 and HEK-293H (Invitrogen) cells were maintained in Dulbecco’s modified Eagle medium supplemented with 10% fetal bovine serum. Cells were transfected using FuGENE 6 (Roche Applied Science) as previously described (18). Knockdown of IQGAP1 by siRNA—IQGAP1 siRNA was engineered by oligonucleotide hybridization as a 19-mer duplex with a 3′-nucleotide spacer loop targeted to base pairs 2061–2079 (siRNA5) and 4589–4597 (siRNA8) of IQGAP1 mRNA (with +1 representing the first nucleotide of the start codon, gttctacgggaagtaattg). Each oligonucleotide pair was

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* The abbreviations used are: MAP, mitogen-activated protein; ERK, extracellular signal-regulated kinase; MEK, MAP kinase/ERK kinase; EGFR, epidermal growth factor; IGF-I, insulin-like growth factor-I; GST, glutathione S-transferase; CHD, calponin homology domain; DSP, di-thiobis(succinimidyl propionate); G3PDH, glyceraldehyde-3-phosphate dehydrogenase; Nt-NTA, nickel nitritotriacetic acid; siRNA, small interfering RNA; HET cells, human embryonic kidney cells; RT, reverse transcription; PVDF, polyvinylidene difluoride. 

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IGQAP1 Modulates the Activity of ERK2

**Plasmid Construction and Preparation of Fusion Proteins—Myc-tagged wild type human IQGAP1, IQGAP1-N, or IQGAP1-C in a pcDNA3 vector was used (14). IQGAP1 lacking the WW domain (IQGAP1-CHD) (amino acids 365–365) were extended to full-length with a 5-min incubation at 72 °C for 23 cycles (at 94 °C for 30 s, 54°C for 30 s, and 68 °C for 1 min). At the conclusion of amplification all products were extended to full-length with a 5-min incubation at 72 °C.

**Reverse transcriptase reactions were performed with a thermal cycler (MJ Research) for 30 min at 50 °C. The reverse transcriptase was inactivated by incubating at 94 °C for 2 min. Subsequently, cDNA was isolated from the cells using RNeasy (Qiagen). RT-PCR primers for IQGAP1 were designed to be within the coding region +4878 to +5438. The sequences and mRNA target sites for the primers were IQGAP1 sense primer 5'-ACCGTTTGGCCAAAAAGAC-3' (+4878 to +4895) and IQGAP1 antisense primer 5'-CTTCCCCGTAAGATTTTGTG-3' (+5436 to +5417). The PCR products were purified from agarose gels with buffer containing 200 mM ATP for 30 min at 30 °C. Phospho-Elk-1 was detected using an antibody specific for phosphorylated ERK (Promega) to detect phospho (active) ERK. The second method employed a p44/42 MAP kinase assay kit. Briefly, active MAP kinase was immunoprecipitated from cell lysates with anti-phospho-p44/42 MAP kinase antibody. Kinase activity was measured by incubating the immunoprecipitates with Elk-1 in kinase buffer (25 mM Tris, pH 7.5, 5 mM MgCl₂, 2 mM dithiothreitol, 0.1 mM Na₃VO₄, and 10 mM MgCl₂) containing 200 μM ATP for 30 min at 30 °C. Phospho-Elk-1 was detected by Western blotting using anti-phospho-Elk-1 antibody.

**Miscellaneous—Densitometry of enhanced chemiluminescence signals was analyzed with UN-SCAN-IT software (Silk Scientific Corp.). Statistical analysis was assessed by Student’s t test with InStat software (GraphPad Software, Inc.). Protein concentrations were determined using the DC protein assay (Bio-Rad).

**RESULTS

**IQGAP1 Binds ERK2—In vitro analysis with pure proteins was conducted to examine a possible interaction between IQGAP1 and ERK2. A GST fusion protein of full-length IQGAP1 was incubated with purified His₅-ERK2, and complexes were isolated with glutathione-Sepharose. Analysis by Western blotting revealed that ERK2 bound to IQGAP1 (Fig. 1A). Binding was specific as no ERK2 was present in the control reaction.**
Experimental Procedures.

His6-ERK2 complexes were isolated and washed as described under "Experimental Procedures." The samples were resolved by SDS-PAGE, transferred to PVDF membranes, and probed with anti-ERK2 antibody. The position of migration of ERK2 is depicted. The data are representative of five independent experiments. B, MCF-7 cells were transiently transfected with p3XFLAG vector (V) or p3XFLAG-ERK2 (ERK2). Equal amounts of protein from cell lysates were incubated with GST-IQGAP1 or GST alone, and complexes were isolated with glutathione-Sepharose. Samples were resolved by SDS-PAGE. After electrophoresis, the gel was cut into two pieces; the top portion (containing IQGAP1) was stained with Coomassie Blue, whereas the bottom half was transferred to PVDF and probed with anti-FLAG antibody. An aliquot of lysate from each sample not subjected to pull-down was also processed by Western blotting (Lysate). The data are representative of three independent experiments.

The samples were resolved by SDS-PAGE, transferred to PVDF membranes, and probed with anti-ERK2 antibody. An aliquot of lysate from each sample not subjected to pull-down was also processed by Western blotting (Lysate). The data are representative of three independent experiments.

To ascertain whether IQGAP1 interacts with ERK2 in a normal cell milieu, MCF-7 cells were transiently transfected with FLAG-ERK2, lysed, and incubated with GST-IQGAP1. ERK2 in cell lysates bound to IQGAP1 (Fig. 1B). The specificity of the binding was validated by the absence of ERK2 from samples that were pulled down with GST alone. Analysis of lysates revealed that FLAG-ERK2 was transfected to the same level in all samples, and the amount of GST-IQGAP1 was equivalent (Fig. 1B).

Effect of EGF on the Interaction of IQGAP1 with ERK2—EGF activates the MAP kinase cascade, enhancing ERK2 activity (1, 29). To determine whether EGF modulates the interaction between IQGAP1 and ERK2, MCF-7 cells were transiently transfected with FLAG-ERK2, and 24 h later EGF was added for 10 min. GST-IQGAP1 or GST alone was added to equal amounts of cell lysate, and protein complexes were extracted with glutathione-Sepharose. Western blotting demonstrated that EGF did not significantly change the amount of ERK2 that bound to IQGAP1 (Fig. 2). Binding was specific because ERK2 was not present in samples that were pulled down with GST alone. Western blotting of lysates showed that FLAG-ERK2 was expressed at the same level in all samples (Fig. 2). The amount of GST-IQGAP1 was equivalent among samples (Fig. 2).

Co-immunoprecipitation of ERK2 with IQGAP1—MCF-7 cells were transiently transfected with FLAG-ERK2 and exposed to a cross-linker, DSP, before lysis. DSP reacts with primary amine groups and cross-links bound proteins. Immunoprecipitation with anti-IQGAP1 antibody revealed that ERK2 bound to endogenous IQGAP1 (Fig. 3A). ERK2 also co-immunoprecipitated with IQGAP1 in the absence of cross-linking with DSP (data not shown). No FLAG-ERK2 was detected in samples immunoprecipitated with non-immune rabbit serum (Fig. 3B). Collectively, these data indicate that IQGAP1 and ERK2 associate in human breast epithelial cells.

Functional Sequelae of the Interaction of IQGAP1 with ERK2—To investigate whether the interaction of ERK2 with IQGAP1 altered ERK signaling, we examined growth factor-stimulated ERK phosphorylation and ERK activity. Analysis was performed in cell lines with overexpression or knockdown of IQGAP1. MCF-7 cells that stably overexpress IQGAP1 (termed MCF/I cells) or empty vector (MCF/V cells) (17, 22) were used. MCF/I cells express 3-fold more IQGAP1 than MCF/V cells (Fig. 4A and Ref. 17). To stably down-regulate IQGAP1 in MCF-7 cells, we used a retroviral vector to integrate a specific siRNA for human IQGAP1 into the genome. The IQGAP1 protein level in these cells (termed MCF/siIQ8 cells) was reduced by 80% (Fig. 4A). The down-regulation was specific for IQGAP1 as levels of ERK2 (Fig. 4A), actin, Cdc42 (30), and Rac1 (data not shown) were not altered. The activity of ERK1 and ERK2 was monitored in these cells by measuring EGF-stimulated changes in ERK1/2 phosphorylation. Lysates were immunoblotted with a phospho-specific anti-ERK antibody that selectively recognizes the doubly phosphorylated, high activity forms of ERK1 and ERK2 (27).
Incubation of serum-starved MCF/V cells with EGF enhanced ERK activity by 4.3 ± 0.27-fold (n = 4) (Fig. 4, A and B). Analysis of the time course of activation showed that EGF-induced phosphorylation of ERKs in MCF-7 cells was maximal at 10–15 min (data not shown). Overexpression of IQGAP1 (in MCF/I cells) reduced by 50% the ability of EGF to stimulate ERK phosphorylation (Fig. 4). Remarkably, EGF was unable to activate ERKs in MCF-siIQ8 cells that have reduced endogenous IQGAP1 (Fig. 4, A and B). As documented by other investigators (31, 32), little phospho-ERK is detected in unstimulated cells. Although this low level of ERK phosphorylation appeared to be somewhat reduced in vehicle-treated MCF/I and

Fig. 3. ERK2 co-immunoprecipitates with IQGAP1. A, MCF-7 cells, transiently transfected with p3XFLAG vector (V) or p3XFLAG-ERK2 (ERK2), were treated with DSP for 10 min. After lysis, equal amounts of protein were immunoprecipitated (IP) with anti-IQGAP1 antibody or non-immune rabbit serum (NIRS). Both unfractionated lysates (Lysate) and immune complexes (IP) were resolved by SDS-PAGE. After transfer to PVDF membranes, the blots were probed with anti-IQGAP1 and anti-FLAG antibodies. Data are representative of three independent experiments. B, untransfected MCF-7 cells were treated with DSP, lysed, and processed as described for panel A. Western blots were probed with anti-IQGAP1 and anti-ERK2 antibodies. Data are representative of three independent experiments.

Fig. 4. IQGAP1 modulates EGF-stimulated ERK activity. A, ERK2 activity was examined in MCF-7 cells stably expressing either vector (MCF/V), IQGAP1 (MCF/I), or siRNA for IQGAP1 (siIQ8). Cells were starved of serum overnight and treated with vehicle or 100 ng/ml EGF for 10 min. Equal amounts of protein from the cell lysates were resolved by SDS-PAGE, transferred to PVDF membranes, and probed with anti-IQGAP1 antibody and an antibody specific for phosphorylated ERK isoforms (Phospho-ERK). The membrane was stripped and reprobed with anti-ERK2 antibody. In addition, immunoprecipitated phospho-ERK activity was determined using Elk-1 as substrate (Phospho-Elk) as described under "Experimental Procedures." Data are representative of four independent experiments. B, the amount of phosphorylated ERK isoforms (Phospho-ERK) was quantified by densitometry and corrected for the amount of ERK2 in the corresponding lysate. Data, expressed relative to the amount of phospho-ERK in vehicle-treated MCF/V cells, represent the means ± S.E. (n = 4). *, significantly different from vehicle-treated MCF/V cells (p < 0.05). **, significantly different from vehicle-treated MCF/V cells (p < 0.001). C, the amount of phospho-Elk was quantified by densitometry. Data, expressed relative to the amount of phospho-Elk in vehicle-treated MCF/V cells, represent the means ± S.E. (n = 3). *, significantly different from vehicle-treated MCF/V cells (p < 0.05). **, significantly different from vehicle-treated MCF/V cells (p < 0.001).
IQGAP1 Modulates the Activity of ERK2

Fig. 5. Altering intracellular IQGAP1 concentrations reduces EGF-stimulated ERK activity. A, MCF-7 cells were transiently transfected with vector (mU6 or pcDNA3), myc-IQGAP1, or siRNAs for IQGAP1 (siIQ5 and siIQ8). Cells were starved of serum overnight and treated with 100 ng/ml EGF for 10 min. Equal amounts of protein from the cell lysates were resolved by SDS-PAGE, transferred to PVDF membranes, and probed with anti-myc antibody (Myc-IQGAP1) (the transfected IQGAP1 is Myc-tagged) and an antibody specific for phosphorylated ERK isoforms (phospho-ERK). The membrane was stripped and reprobed with anti-IQGAP1 and anti-ERK2 antibodies. Data are representative of three independent experiments. B, the amount of phosphorylated ERK isoforms (phospho-ERK) was quantified by densitometry and corrected for the amount of ERK2 in the corresponding lysate. Data, expressed relative to the amount of phospho-ERK in cells transfected with pcDNA3 vector, represent the means ± S.E. (n = 3). *p < 0.001, significantly different from pcDNA3 vector.

MCF-siIQ8 cells (Fig. 4, A and B), the reduction was not statistically significant.

Phosphorylated ERK is very widely used as a measure of active ERK, but it may not always reflect kinase activity (33). To validate our findings, activity of ERK1/2 was also measured directly in a kinase assay. Phosphorylated ERK1/2 was immunoprecipitated from MCF-7 cells, and kinase activity was analyzed by its ability to phosphorylate Elk-1. EGF increased Elk-1 phosphorylation by 6.2 ± 1.0-fold (n = 3) in MCF/V cells (Fig. 4, A and C). In MCF/F cells EGF-stimulated phosphorylation of Elk-1 was 50% lower than in MCF/V cells. The magnitude of reduction in the ability of EGF to enhance Elk phosphorylation in MCF/F cells was the same as the reduction in phospho-ERK in these cells. Moreover, EGF was unable to significantly increase Elk-1 phosphorylation in MCF-siIQ8 cells (Fig. 4, A and C). These results corroborate our findings obtained with the phospho-ERK antibody, thus validating phosphorylated ERK as an indicator of ERK activity in our assay system. Together, our results suggest that an optimal level of intracellular IQGAP1 is required for maximal activation of ERK1 and ERK2 by EGF.

The effect of IQGAP1 on EGF-stimulated ERK activity was confirmed in transiently transfected cells. Knockdown of IQGAP1 by transient expression of siRNA 8 in MCF-7 cells reduced IQGAP1 protein expression by 50% (Fig. 5A). ERK phosphorylation in these cells was 70% lower than in vector-transfected cells (Fig. 5, A and B). In contrast, neither siRNA 5, which does not reduce IQGAP1 (Fig. 5A), nor the mU6 pro vector used to introduce the siRNAs, had any effect on ERK phosphorylation. Analogous to the results with MCF/F cells, transient overexpression of IQGAP1 in MCF-7 cells significantly reduced activation of ERK1 and ERK2 by EGF (Fig. 5, A and B).

Effects of Knockdown of IQGAP1 by siRNA on the Levels of IQGAP1 mRNA and Components of the MAP Kinase Signaling Pathway—Two complementary strategies were used to assess the specificity of siRNA for IQGAP1. In the first approach mRNA was quantified by RT-PCR. Stable integration of siRNA 8 in MCF-7 cells substantially reduced mRNA for IQGAP1 (Fig. 6A). Transient transfection of siRNA 8 similarly reduced IQGAP1 mRNA. In contrast, cells transfected with siRNA 5, which does not reduce IQGAP1 (Fig. 5A), nor the mU6 pro vector used to introduce the siRNAs, had any effect on ERK phosphorylation. Analogs to the results with MCF/F cells, transient overexpression of IQGAP1 in MCF-7 cells significantly reduced activation of ERK1 and ERK2 by EGF (Fig. 5, A and B).

The specificity of siRNA for IQGAP1 was further validated by Western blotting. Stable knockdown of IQGAP1 protein by siRNA 8 had no effect on the amount of Raf, MEK, or Ras protein in MCF-siIQ8 cells (Fig. 6B). Similarly, transient expression of siRNA 8, which reduces IQGAP1 protein, had no significant effect on the protein levels of the components of the MAP kinase pathway (Fig. 6B). The uniform amount of β-tubulin among samples documents that equal amounts of protein lysate were loaded. Together with the lack of an effect on ERK2 levels (see Figs. 4 and 5), these data validate that siRNA 8 is
Identification of the ERK2 Binding Domain on IQGAP1—

The region of IQGAP1 to which ERK2 binds was investigated using portions of IQGAP1 labeled with biotin-S-methionine. Selected constructs of IQGAP1 (depicted in Fig. 8A) were labeled with biotin-S-methionine in a reticulocyte lysate and incubated with His$_{6}$-ERK2. Constructs that bound to His$_{6}$-ERK2 were isolated by Ni$_{2+}$ affinity chromatography, resolved by SDS-PAGE, and identified by autoradiography. Analogous to the isolation of ERK2 by full-length GST-IQGAP1, biotin-S-methionine-labeled full-length IQGAP1 bound to His$_{6}$-ERK2 (Fig. 8B, upper panel). No IQGAP1 bound to Ni$_{2+}$ beads alone (Fig. 8B, first lane). Examination of the two halves of IQGAP1 revealed that only the N-terminal half (amino acid residues 1–863) bound to His$_{6}$-ERK2; no interaction between the C-terminal half of IQGAP1 and ERK2 was detected. The amount of His$_{6}$-ERK2 in each sample was essentially identical (data not shown).

To narrow the binding site, the N-terminal portion of IQGAP1 was divided into two equal halves, termed N1 and N2 (Fig. 8A). His$_{6}$-ERK2 bound exclusively to N2 (Fig. 8B), implying that the binding domain was between residues 432 and 863 of IQGAP1. Subsequently, all of the identified protein interaction domains in the N-terminal half of IQGAP1 were deleted (Fig. 8A). Analysis of binding to His$_{6}$-ERK2 revealed that the absence of the CHD (amino acids 37–265) and IQ, which lacks the CHD (amino acids 643–744), completely eliminated ERK2 binding (Fig. 8B). These data reveal that the region of IQGAP1 containing amino acids 643–744 is necessary for ERK2 binding.

To determine whether amino acids 643–744 are sufficient for ERK2 binding, a GST fusion protein of these residues (termed GST-WW) was constructed. Incubation of pure proteins revealed that His$_{6}$-ERK2 bound specifically to GST-WW (Fig. 8C). Less His$_{6}$-ERK2 associated with GST-WW than with full-length IQGAP1 (Fig. 8C), not withstanding that the amount of GST fusion proteins was equivalent among samples (data not shown). Therefore, the affinity of ERK2 binding to the WW domain is lower than that to full-length IQGAP1, implying that residues outside amino acids 643–744 are likely to contribute to the interaction. Nevertheless, these findings indicate that the region including the WW domain of IQGAP1 is both necessary and sufficient for ERK2 binding.

**DISCUSSION**

IQGAP1 associates with multiple proteins including calmodulin, Cdc42, Rac1, actin, β-catenin, E-cadherin, CLIP-170, and S100B (9, 10). By interacting with these targets, IQGAP1 participates in many cellular functions including cell-cell adhesion, transcription, cytoskeletal architecture, and selected signaling pathways (9). In this study we add ERK2 to the burgeoning array of IQGAP1 targets. We document a direct in vitro interaction between purified IQGAP1 and ERK2. Moreover, co-immunoprecipitation revealed that endogenous IQGAP1 binds to endogenous ERK2. This in vivo interaction has functional significance because manipulation of intracellular IQGAP1 concentrations significantly impaired the ability of both EGF and IGF-1 to activate ERK. In contrast, overexpression of IQGAP1ωWW, which lacks the ERK2 binding region, did not attenuate EGF-stimulated activation of ERK.

On the basis of the documented association of ERK with the CHDs of calponin and α-actinin (21), we predicted that ERK2 would bind to the CHD of IQGAP1. Experimental evidence did not support this hypothesis. Although ERK2 bound to the N-terminal half of IQGAP1, which includes the CHD, deletion of the CHD did not attenuate the interaction of ERK2. In contrast, deletion from IQGAP1 of amino acids 643–744, which includes the WW domain, completely eliminated ERK2 binding. Moreover, ERK2 specifically bound a GST fusion peptide comprising amino acids 643–744 of IQGAP1, indicating that this region is necessary and sufficient for the association of ERK2. The affinity of ERK2 for GST-WW is lower than its affinity for full-length IQGAP1, suggesting that additional residues outside this region contribute to binding. Another possibility is that the peptide does not adopt the same conformation as that found in the native protein, thus weakening the interaction. The WW protein interaction motif, which has been identified in several signaling and structural proteins, binds to proline-rich regions or phosphoseryl/phosphothreonine residues on target proteins (35). However, ERK2 does not contain a proline-rich region. Moreover, IQGAP1ΔIQ, which lacks the distal tryptophan of the WW motif, binds ERK2. It seems likely, therefore, that residues outside the short WW motif participate in the interaction with ERK2. No protein has been identified that binds exclusively to the WW domain of IQGAP1.
FIG. 8. Identification of amino acid residues of IQGAP1 necessary for binding to ERK2 in vitro. A, schematic representation of IQGAP1 constructs. Full-length and deletion mutants of IQGAP1 are depicted. The identified protein interaction motifs and the specific amino acid residues absent from each mutant are indicated. WW, poly-proline binding domain; IQ, four tandem calmodulin binding motifs; GRD, Ras-GAP-related domain; IQGAP1-N, N-terminal half of IQGAP1; IQGAP1-C, C-terminal half of IQGAP1. B, [35S]methionine-labeled pcDNA vector (V), wild type IQGAP1 (WT), IQGAP1-N (N), IQGAP1-C (C), IQGAP1-N1 (N1), IQGAP1-N2 (N2), IQGAP1WW (WW), IQGAP1ΔCHD (ΔCHD), and IQGAP1ΔIQ (ΔIQ) were incubated with equal amounts of His$_6$-ERK2. Wild type IQGAP1 was also incubated with Ni$^{2+}$-NTA resin (first lane from left) alone as control. Complexes were isolated with Ni$^{2+}$-NTA-agarose beads and resolved by SDS-PAGE. Gels were dried and processed by autoradiography (His$_6$-ERK2 pull-down, upper panel). In addition, an aliquot of [35S]methionine-labeled TNT product (equivalent to 10% of the amount that was subjected to pull-down) was resolved by SDS-PAGE, dried, and processed by autoradiography (Input, lower panel). Data are representative of three independent experiments. C, purified His$_6$-ERK2 was incubated with GST-IQGAP1 (IQGAP1), GST-IQGAP1-C (C), GST-WW (WW), or GST alone bound to glutathione-Sepharose. Complexes were isolated and washed as described under “Experimental Procedures.” The samples were resolved by SDS-PAGE, transferred to PVDF membranes, and probed with anti-ERK2 antibody. The position of migration of ERK2 is depicted. The data are representative of three independent experiments.
Changing the intracellular IQGAP1 concentration reduced growth factor-stimulated phosphorylation of ERK. Interestingly, both increasing IQGAP1 levels and knockdown of endogenous IQGAP1 had the same results, namely impairment of stimulation of ERK by EGF or IGF-I. In addition, altering IQGAP1 levels reduced the kinase activity of ERK, as measured by its ability to phosphorylate the substrate Elk-1. Importantly, overexpression of IQGAP1ΔWW, which is unable to bind ERK2, did not modulate the ability of EGF to activate ERK. Together, these findings suggest that an association with IQGAP1 is necessary for modulating the activity of ERK2. It is possible that an appropriate stoichiometric ratio of IQGAP1 to ERK2 is necessary for maximal activation of ERK2. When IQGAP1 is in excess or insufficient, optimal signal integration does not occur.

Our results need to be interpreted in the context of what is known about MAP kinase signaling. In yeast, specificity in MAP kinase signaling is attained predominantly by scaffolding proteins that determine which MAP kinase pathway is trig-

erred in response to a given stimulus. Similarly, mammalian cells contain scaffolding proteins like kinase suppressor of Ras (KSR), connector enhancer of KSR (CNK), Sur8, MEK partner 1 (MP1), and Raf kinase inhibitor protein (RIP), that modulate signaling from Raf1 to MEK1/2 and ERK (8, 36, 37). Other mechanisms that confer MAP kinase signaling specificity in mammalian cells include selective phosphorylation of scaffolds, sequestering the kinases, and restricting kinases to distinct subcellular pools (38).

A common theme in growth factor signaling cascades is the induction of specific intracellular protein-protein interactions as a consequence of activation of growth factor receptors. This model does not apply to the findings reported in our study because EGF, a well known activator of ERKs (1, 29), did not alter the interaction between IQGAP1 and ERK2. This observation implies that ERK2 binding to IQGAP1 is constitutive and independent of ERK phosphorylation.

Several possible models could explain our data. The previously described dimerization of IQGAP1 (39) may be relevant to the effects we observed. Dimerization of IQGAP1 may be necessary for its functional association with ERK2. Conceivably, both increasing and decreasing intracellular IQGAP1 concentrations could reduce the number of functional dimers, thereby attenuating growth factor-stimulated ERK activity. A second possibility is that IQGAP1 may function as a scaffold in the MAP kinase pathway. Although this mechanism is consistent with the data, no evidence exists that IQGAP1 binds any of the upstream activators of ERK2, which is required to support this hypothesis. IQGAP1 binds diverse proteins (e.g., Ca^2+/-calmodulin, Cdc42, and E-cadherin) and integrates the activity of multiple signaling pathways (9, 10). For example, via its interaction with IQGAP1, calmodulin regulates Cdc42 (14) and E-cadherin (18) function. These observations suggest a third model where IQGAP1 acts as a node to enable other signaling pathways to converge on the MAP kinase pathway, thereby altering ERK activity. Finally, IQGAP1 may influence MAP kinase signaling by regulating the subcellular location of ERK2. Select stimuli, such as altering intracellular Ca^2+ concentration (15) or calmodulin function (18), redistribute the subcellular location of IQGAP1. By this mechanism, IQGAP1 may change the access of ERK2 to regulatory kinases and phosphatases, leading to modified ERK2 function.

In the current study we identified a novel association between IQGAP1 and ERK2. This interaction, documented both in vitro and in cells, was independent of EGF. Both overexpression and specific knockdown of endogenous IQGAP1 significantly impaired growth factor-stimulated ERK activities. These data suggest that an optimal level of intracellular IQGAP1 is necessary for maximal activation of ERK by EGF.

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