The Small GTPases Ras, Rac, and Cdc42 Transcriptionally Regulate Expression of Human Fibroblast Growth Factor 1*

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Four distinct promoters (1A, 1B, 1C, and 1D) of fibroblast growth factor 1 (FGF1), spaced up to 70 kilobase pairs apart, direct the expression of alternatively spliced transcript variants (FGF1A, -1.B, -1.C, and -1.D) that encode FGF1. These FGF1 transcripts can be detected in cultured cells as well as in normal and diseased tissues. These transcripts are differentially regulated in a cell-specific manner. To further delineate the biological function of multiple promoter usage by a single gene, we investigated the transcriptional regulation of these promoters by defined signaling pathways associated with cell proliferation and cell survival. Here we show a specific association of two of the FGF1 promoters, 1C and 1D, with signaling cascades of the Ras superfamily of GTPases. A serum-response element, comprised of the Ets and CarG motifs, present in promoter 1D was shown to be the target of distinct signaling cascades; the Ets motif target of Ras, Rac1, and Cdc42 regulation; and the CarG motif target of de novo protein synthesis-independent cascade. Ras and Rac1 also activated the FGF2 promoter. Further, the transcription factor Ets2 synergistically activated FGF1 gene, but not FGF2, in a Ras- and Rac1-dependent signaling pathway. In support of these conclusions high levels of intracellular FGF1 were detected in cells undergoing cytokinesis. Altogether, our results suggest that FGF1 may play a fundamental role in cell division, spreading, and migration, in addition to cell proliferation.

Fibroblast growth factor 1 (FGF1) is a potent angiogenic and cell survival factor (1). Its 155-amino acid single chain polypeptide is encoded by three protein-coding exons. However, the FGF1 gene organization, spanning more than 100 kbp, as well as expression pattern of FGF1 transcripts, is indeed complex because of the existence of at least four 5′-untranslated exons (2). Four different FGF1 transcripts (FGF1A, -1.B, -1.C, and -1.D), varying only in the 5′-untranslated region, originate from four distinct promoters (1A, 1B, 1C, and 1D) (2–4). These promoters are differentially regulated in a cell- and tissue-specific manner (4–7). FGF1.A transcript was detected exclusively in the kidney (2, 3). High FGF1 promoter 1B activity was detected in a glioblastoma cell line U1240MG with promoter construct extending up to nucleotide −540 from the transcription start site. This activity was attributed to a 23-bp cis-element (~489 to ~467). A 37-kDa protein, designated p37βα, was found to associate within this sequence and postulated to positively regulate expression of FGF1.B transcript in the brain (5–6). In contrast, a basic helix-loop-helix protein, E2–2, negatively regulates the 1B promoter (7). Also, FGF1 promoter 1C construct extending up to ~1614 from the transcription start site displayed activity in PC-3 prostate carcinoma cells and serum inducibility in MDA-MB-231 breast carcinoma cells. The promoter contained cis-elements that included activator protein 1, activator protein 2, and Sp1. These elements showed specific association with nuclear proteins (8). In this study, we have further dissected the signaling cascades involved in FGF1 gene regulation. Here we report molecular mechanisms that explain the differential regulation of these promoters, particularly promoters 1C and 1D, and further provide insight into the biological role of multiple FGF1 promoters.

EXPERIMENTAL PROCEDURES
Southern Blot Analysis

This analysis was performed using standard protocol (9). Tissue and cells expressing variant FGF1 mRNAs were used, i.e. high levels of FGF1.A in human kidney and weak levels in human embryonic kidney 293, high levels of FGF1.B in U-1240 MG and FGF1.C and -1.D in saphenous vein smooth muscle cells and embryo lung fibroblasts (M426; 2–4). Radiolabeled DNA probes specific for the 1A, 1B, 1C, and 1D promoters were: 2.4-kbp EcoRI-EcoRI containing the 1A promoter, 0.89-kbp HindIII-XhoI containing the 1B promoter, 1.2-kbp BgII-BgII containing the 1C promoter, and 1.2-kbp EcoRI-BgII containing the 1D promoter.
digestion with Xhol and religation. DNA sequencing confirmed the authenticity of this construct. A 1.2-kbp EcoRI-BgII genomic DNA fragment containing the 1D promoter was linearized at EcoRV (present in the vector pBluescript KS(+)) upstream of the EcoRI site) and then digested with NcoI to release a 900-bp fragment. This fragment was ligated to the −150 to +40 1D fragment in pGL2-Basic that had been digested with SmaI (present in the multicloning site, upstream of −150) and NcoI (present within the −150 to +40 region of 1D). This generated the −985 to +40 1D luciferase reporter.

The −580 to +40 1D luciferase reporter construct was generated by digesting the 1.2-kbp EcoRI-BgII genomic DNA fragment containing the −1D promoter region with Hpal and NcoI (−545 to −82) to release a 460-bp fragment. The gel-purified fragment (GeneClean II, Bio101) was ligated to the −150 to +40 1D fragment in pGL2-Basic digested with NheI (present in the multicloning site, upstream of −150), blunt-ended with dNTPs and Klenow fragment, and subsequently digested with NcoI (present with the −150 to +40 region). This construct was propagated in the routinely used Escherichia coli strain DH5α (Life Technologies, Inc.) cells resulted in deletion in the plasmid DNA. Utilization of STBL2™, suitable for unstable DNA sequences (12), eliminated the problem of deletion, and the correct, intact DNA was recovered.

The −545 to +40 construct was linearized at SacI (present in the multicloning region, upstream of Ets2) and then digested with Bst XI site for CArG-MUT and an PstI site. This manipulation allowed enrichment of the Ets transcription factors on the truncated promoter constructs Ets WT/CArG-MUT (−179 to +40), and Ets MUT/CArG WT (−183 to +40) showed a similar trend compared with the SRE mutations in the context of the (−985 to +40) construct. The expression plasmid EFplink was used to make up the total amount of DNA to 8 µg.

Ras responsiveness was initially determined by looking at the difference in reporter activities without and with effector Ras (expression plasmid pDCR1 or pDCR2, or pDCR3) in the presence of Ras (0.125 or 0.5 µg) with empty expression plasmid. The pDCR1 plasmid gave the best results and was used in all subsequent experiments.

GTP/GDP-binding Protein Regulation Studies—Cells were incubated in 0.5% serum for a total of 48 h with a medium change once at 24 h. Cells were harvested, and luciferase activity and β-galactosidase activity were determined. For the Ras protein studies, 1 µg of pCH110, 4 µg of reporter, and 1 or 3 µg of EFHoA-V14, Rac1-V12, and Cdc42hs-V12 plasmids each, were co-transfected, and specific dose effects were noted. The truncated promoter constructs Ets WT/CArG MUT (−179 to +40), and Ets MUT/CArG WT (−183 to +40) showed a similar trend compared with the SRE mutations in the context of the (−985 to +40) construct. The expression plasmid EFplink was used to make up the total amount of DNA to 8 µg.

Other Plasmid Constructs

EFHoA-V14, Rac1-V12, and Cdc42hs-V12—The activated Rho proteins, including RhoA-V14, Rac1-V12, and Cdc42hs-V12, were expressed via the EFlink vector that contains the human elongation factor 1α promoter (21). pCH110—The β-galactosidase-expressing vector, used generally in transient transfections as an internal control, comprises the E. coli lacZ gene driven by the simian virus 40 early promoter (15).

HSV-TK—This construct is the pGL2-Basic luciferase reporter driven by the herpes simplex virus-thymidine kinase (HSV-TK) basal promoter (−89 to +1004) (14). 5X SRE—This luciferase reporter construct contains five copies of the c-fos promoter region harboring the SRE, comprised of Ets and CarG elements (5′-AGG ATG TCC ATA TTA GGA CAT CT-3′) pSRE-Luc, Stratagene).

FGF2 Luciferase—pPF2CAT (−1800 to +175; 16) was digested with SacI and EcoRI digestion of both BglII and Klenow fragment, and then digested with Xhol. The pGF2 promoter fragment was ligated into pGL2-Basic digested with SmaI and Xhol.

**Transient Transfections**

All plasmid DNA for transfections were prepared using Qiagen plasmid purification kit (Qiagen, Inc.). NIH/3T3 cells (2 × 10⁵) were seeded on 60-mm plates and allowed to grow for at least 24 h before transfection. Transfections were performed using 30 µl of liposomal transfer reagent DOTAP (Roche Molecular Biochemicals) and DNA in a total volume of 200 µl (20 mM HEPEs, 150 mM NaCl, pH 7.4). This mixture was allowed to incubate for 30 min at room temperature to facilitate stable complex formation between DNA and DOTAP. This was followed by transfer to the cells in 1.5 ml of culture medium containing 10% CS. After incubation at 37 °C for at least 20 h, the medium was removed and replaced with fresh medium containing 0.5% serum. Cells were subsequently treated as described above.

**Cycloheximide Induction Studies—**Cells were incubated for 34 h followed by a medium change. The fresh medium comprised of either 0.5% serum only, 10% serum only, 0.5% serum plus cycloheximide (10 µg/ml tissue culture grade in ethanol; Sigma), or 10% serum plus cycloheximide. After 12 h of incubation the medium was removed, cells were washed twice with fresh medium containing 0.5% serum only, followed by an additional incubation of 2 h in medium with 0.5% serum (cycloheximide-washout). The cells were washed twice with phosphate-buffered saline and subsequently harvested using lysis reagent (Promega). The reporter activity was determined using a luminometer (Lumat LB 9501, EG & G Berthold, Berthold Systems Inc., Pittsburgh, PA). The β-galactosidase activity of the internal control was measured using the same instrument using a chemiluminescent assay (Galacto-Light™, Tropix, Bedford, MA). Transfections were performed in duplicate or in triplicate, and the data were expressed as the ratio of reporter activity versus β-galactosidase activity. One µg of pCH110 and 4 µg of reporter were used for all experiments. Sera from various companies, including Life Technologies, Inc., BioWhittaker, and Hyclone, were tested in the presence of pHSV-TK (Cosmic Ho) and subsequent screening of each serum gave the best results and was used in all subsequent experiments.
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**RESULTS**

**FGF1 Promoter 1D Is Uniquely Superinduced by Serum/ Cycloheximide**—To rule out the possibility of gene reorganization as a potential mechanism for cell-specific expression and regulation, the critical regulatory regions were examined by Southern blot analysis using probes specific for each of the four FGF1 promoters (Fig. 1). The analysis was performed on genomic DNA from tissues and cells expressing variant FGF1 transcripts. No rearrangement was noted in any of the genomic DNA analyzed (Fig. 1). A polymorphism was noted in the promoter 1D region in DNA isolated from smooth muscle cells (Fig. 1). These data suggested that deletion or rearrangement of regulatory regions, as seen in immunoglobulin gene rearrangement, was not the mechanism of regulation. These data further suggested that the differential regulation of FGF1 mRNAs was probably because of cell-specific transcriptional regulation. To test this hypothesis, we pursued our previous observation of FGF1.D mRNA superinduction by serum in the presence of the protein synthesis inhibitor cycloheximide noted in smooth muscle cells and fibroblasts (4). This superinduction was reminiscent of a phenomenon previously reported for c-fos and Ｇ-actin and was mediated via a cis element identified as the CarG box, also known as the core element of the SRE (24). The functionality of promoter 1D was measured using luciferase reporter activity in transient transfection assays. In comparison to FGF1 promoters 1A, 1B, and 1C, promoter 1D was uniquely superinduced and behaved similarly to the endogenous gene (Fig. 2, CS/cycloheximide versus Starved and CS). Furthermore, heat inactivation of serum at 56°C for 30 min and repeated freezing-thawing of serum stocks dramatically affected the extent of this inducibility (data not shown). The data showed that (i) cycloheximide alone was not the inducing agent, and (ii) cycloheximide inducibility may depend on serum component(s) that are heat labile. Altogether, these observations supported the notion of a transcriptional enhancement of promoter 1D activity with serum in the presence of cycloheximide via a signaling pathway activated by serum component(s) that are heat labile. Altogether, these observations supported the notion of a transcriptional enhancement of promoter 1D activity with serum in the presence of cycloheximide via a signaling pathway activated by serum component(s) and independent of de novo protein synthesis. Because CS was unable to superinduce promoter 1D, negative factors likely were involved in promoter 1D expression. That these factors were labile was demonstrated by the induction of 1D by cycloheximide alone. Thus, the combination of CS and cycloheximide elicits the phenomenon of superinduction. FGF1 Promoter 1D Superinducibility Is Mediated by a CarG Motif—To identify the promoter 1D cycloheximide inducible element, reporter constructs with various 5′-truncations were tested in transient transfections. These experiments narrowed down the element to nucleotides −277 and −150, as reflected by a decrease in promoter inducibility by 43% (Fig. 3A). Sequence analysis of promoter 1D revealed a putative SRE comprising a CarG element between −164 and −155, along with an adjacent Ets element between −172 and −167. Thus, the SRE was a good candidate for site-directed mutagenesis. Mutagenesis of the SRE was based on previously described contact points of Ets serum response factors with the c-fos SRE (25). Indeed, mutation of the CarG element, but not the adjacent Ets

**Fig. 1. Southern blot analysis of FGF1 promoter regions.** The top panel shows the gene organization of the FGF1 gene with each of the four promoters indicated. The three coding exons are indicated as filled boxes. The numbering (in kbp) indicates the distance of each upstream, untranslated exon in reference to the coding exon 1, taken as 0. The broken lines indicate alternative splicing of the 5′-untranslated exons to the coding exons, giving rise to variant FGF1 mRNAs (FGF1.A, -1.B, -1.C, and -1.D). The expected size fragments (in kbp) for 1A to 1D are indicated by arrowheads, along with a polymorphic band for 1D (+), ~12 kbp.

or 0.5 μg). Cells were harvested, and luciferase activity and β-galactosidase activity were determined. For all transfection experiments, at least two independent experiments in duplicate or triplicate were performed.

**Synchronization and Immunohistochemistry of NIH/3T3 Cells**

NIH/3T3 cells were synchronized using serum starvation as described previously (23). Briefly, cells were seeded on 8-chamber slides at a density of 5 × 10⁴/chamber, and allowed to grow for four days to confluence. On day 5, confluent cells were starved with Dulbecco’s modified Eagle’s medium containing 0.5% CS for two days. CS was added to a final concentration of 20% on day 7. At indicated intervals after serum stimulation (0, 2, 6, 16, and 24 h), cells were fixed with ice-cold acetone for 2 min and prepared for immunohistochemistry. The slides were treated with 3% H₂O₂ to block endogenous peroxidase activity. After serum stimulation (0, 2, 6, 16, and 24 h), cells were fixed with ice-cold acetone for 2 min and prepared for immunohistochemistry. The slides were treated with 3% H₂O₂ to block endogenous peroxidase activity. After incubation with secondary antibody at room temperature for 45 min and phosphate-buffered saline rinses (3 × 5 min), the cells were reacted with the ABC reagent for 45 min at room temperature. The color reaction was performed using AEC chromagen (Vector Labs), and the cells were counted using a de novo counter.

**RESULTS**

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**Fig. 2. Cycloheximide inducibility of FGF1 promoters.** Four FGF1 promoters (1A (~826 to +77), 1B (~531 to +31), 1C (~786 to +88), and 1D (~985 to +40) and HSV-TK basal promoter (TK, ~80 to +50) driving the luciferase reporter gene were individually tested using transient transfections. The promoterless pGL2-Basic (pGL2) vector was also used as a control for background activity. The average of triplicate sets with standard error is shown.
site, significantly attenuated (up to 74%) the serum/cycloheximide effect giving activity comparable to the −150 promoter construct (Fig. 3). Thus, a signaling pathway independent of de novo protein synthesis was activated by serum component(s) and was linked to FGF promoter 1D via the CArG box component of a serum response element. Consequently, cycloheximide alone leads to increased promoter activity (see Fig. 2, 1D, cycloheximide versus Starved). The CArG element plays an important role in transcription in several muscle-specific promoters (25). To test the function of FGF1 promoter 1D CArG element in muscle-specific expression, we tested the activity of FGF1 promoter 1D in proliferating myoblasts. The results indicated that promoter 1D was indeed active in this cell type. Promoter 1D mutants showed that the SRE played a significant role in promoter activity, with the CArG element displaying a more significant role than the adjacent Ets site; compared with wild type, there was a 79% drop in promoter activity with CArG MUT/Ets WT versus a 34% drop for Ets MUT/CArG WT. Altogether, these data suggested that the SRE played a crucial role in regulating promoter 1D activity.

**Fig. 3.** Identification of the promoter 1D cycloheximide inducible cis element in NIH/3T3 cells. Transfections were performed as described under “Experimental Procedures,” with 10% serum, in the absence or presence of cycloheximide (−/+), A, the wild-type 5'-truncated FGF1D promoter constructs (−985 to +40, −545 to +40, −339 to +40, −277 to +40, and −150 to +40) were tested. The results allowed localization of the cycloheximide inducible element to nucleotides −277 and −150. B, site-directed mutagenesis of CArG box between −164 and −155 (CArG MUT) and not the flanking Ets site (between −172 and −167, Ets-MUT) leads to a loss of cycloheximide inducibility. These mutations were introduced in the −985 to +40 reporter construct. The average of duplicate sets with standard error is shown. The relative promoter activity is shown with the activity of (WT) −985 to +40 construct taken as 100%. The pGL2-Basic vector was used as a control for background activity.

**Fig. 4.** Rho family GTPases regulate FGF1 gene expression in NIH/3T3 cells. A, activated Rho proteins, Rac1-V12 and Cdc42-V12, are involved in promoter 1D regulation. FGF1 promoter regions tested were: 1A (−826 to +77), 1B (−831 to +31), Δ1C (−786 to +88), 1C (−1601 to +88), or 1D (−985 to +40). Data using 3 μg of effector plasmids are shown. B, mutation of the SRE Ets site of the 1D promoter region (−985 to +40) impairs Rac1 and Cdc42 regulation. Here the relative reporter activity (in percent) of the WT sequence is compared with the activity of the reporter with the CArG mutation (CArG-MUT) or the Ets mutation (Ets-MUT) in response to 3 μg of effector plasmid. The average of duplicate or triplicate sets with standard error is shown. C, Rho protein gene regulation of FGF2, c-fos SRE (five copies in tandem array), and (D) 0.75-kbp c-fos promoter containing a single copy of the SRE. The pGL2-Basic vector was used as a control for background activity.

**FGF2 promoter** (Fig. 4C). Interestingly, the c-fos SRE in the context of its own promoter showed only a weak response to activated GTPases (Fig. 4D). The result underscores the robustness of the FGF1 and FGF2 promoter responses. Further, Ras was involved in regulation of both promoters 1D and 1C, as well as the FGF2 promoter (Fig. 5A). We also examined the bladder carcinoma cell line J82 overexpressing oncogenic Ras (stable transfections). The results showed a 3–4-fold increase in steady state levels of FGF1D mRNA compared with control; another bladder carcinoma cell line EJ also expressed detectable levels of 1D mRNA as determined by RNase protection assays (data not shown). High levels of FGF1C mRNA were not detected in these cells, which is consistent with the kinetics...
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Pathway mutants; RasV12C40 is deficient in MAP kinase pathway signaling but efficient in inducing membrane ruffling, whereas RasV12S35 is defective in inducing membrane ruffling but effective in inducing the MAP kinase pathway signaling (18). Both Ras mutants failed to induce FGF1C, -1D, FGF2, and c-fos SRE promoters. Unexpectedly, co-transfections with both RasV12C40 and RasV12S35 failed to rescue gene expression (data not shown). These data suggested that in NIH/3T3 cells, unlike in rat embryo fibroblast cells (18), both amino acid residues 35 and 40 of Ras are required in cis for proper effector interaction(s).

Divergent Signaling Pathways Converge on the SRE of FGF1 Promoter 1D—We examined the involvement of Mek in the fetal bovine serum/cycloheximide superinduction of promoter 1D by using the dominant inhibitory Mek1 mutant (Mek WT/ Ser222 to Ala (S222A)) and wild type constructs (Mek WT). The results showed that Mek WT had no inhibitory effect on the superinduction with the Mek WT displaying up to 17% inhibition, when compared with vector control (taken as 100%). A pitfall in this experiment may be the use of cycloheximide itself, i.e., cycloheximide may confound these results by interfering with the expression of MUT plasmids. To address this possibility, we repeated the experiments with a specific Mek 1 blocker PD98059 (26). As a control, 5X SRE-driven luciferase reporter was also used. The Ets motif in this c-fos SRE is the target of regulation by the MAP kinase pathway. It is also in sharp contrast to the c-fos regulation by Rac1 and Cdc42 GTPases that require the c-ArG/serum response factor for promoter activation by the Ras-Raf- MAP kinase pathway. It was also observed that the adjacent Ets site (14). Collectively (i) the Ets site-binding protein is a target of regulation and (ii) the Ets and c-ArG-binding protein cooperation does not appear to be important. This regulation is unlike the previously proposed model of a ternary complex formation at the SRE that requires Ets factor binding along with the serum response factor for promoter activation by the Ras-Raf MAP kinase pathway. It is also in sharp contrast to the c-fos regulation by Rac1 and Cdc42 GTPases that require only the c-ArG/serum response factor interaction and not the adjacent Ets site (14).
The IC$_{50}$ of PD98059 for Mek1 activity is 2 μM. Also, the 5X SRE control showed a similar trend, with 6.6 and 26.6% inhibition (compared with control with vehicle, taken as 100%). This effect was consistent with the accepted role of the CArG motif and not the Ets motif, in participating in the superinduction (24, 25). We also examined the effect of SB202190, a specific inhibitor of the p38α and p38β stress-activated protein kinase (28). When used at 4 μM (14-fold above IC$_{50}$ of 280 nM), this inhibitor showed no effect on the superinduction. Altogether, these results demonstrate that the Ets and CArG motifs of promoter 1D are targets of divergent signaling pathways.

The Transcription Factor Ets2 Synergizes Ras and Rac1 Responsiveness of FGF1 Promoters—The involvement of the Ets family of transcription factors was directly examined, specifically Ets2 and PEA3. The Ras-Raf MAP kinase-dependent selective phosphorylation of a conserved threonine in the pointed domains of Ets1 and Ets2 enhances their trans-activation potential (21, 22). When transfected singly, Ets2 (0.5 μg) provided a 2.7- and 1.7-fold induction for the FGF1 promoter 1C and 1D, respectively (Fig. 5, A and B). The cells at indicated time points following serum stimulation were reacted to antibodies to FGF1 and counterstained with hematoxylin. The quantification of FGF1 positive cells is shown in Table I.

Collectively, these results underscore the biological significance of Ets protein(s) plus Ets2 (Fig. 6, A–D). This effect was, however, not observed for RasV12 (0.5 μg) and Rac1 (0.5 μg) provided a 8.3- and 4-fold induction for promoters 1C and 1D, respectively (Fig. 5, C and D). This trend continued up to 24 h after serum addition (Fig. 7, D). However, at 16 h after serum addition, FGF1 was detected specifically during the cytokinesis phase of mitosis (Fig. 7C), with as high as 98.3% cells expressing FGF1 (Table I). This trend continued up to 24 h after serum addition (Fig. 7D), although the percentage of FGF1 positive cells decreased slightly (90.5%).

**DISCUSSION**

In this study we have identified two of the four FGF1 promoters (1C and 1D) to be specifically under the regulation by the Ras superfamily GTPases Ras, Rac1, and Cdc42. These findings combined with our previous reports showing that the brain-specific FGF1 promoter 1B is under the control of other transcription factors, E2–2 and p37brn (6, 7), provide definitive evidence for a single gene to be regulated by distinct signaling mechanisms through different promoters. This type of regulation sets a new paradigm for gene expression. The molecular basis of the FGF1 gene expression, which we uncovered in this report, further emphasizes the necessity in fine tuning the pleiotropic effects exerted by FGF1.

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**Fig. 7.** *FGF1* expression during cytokinesis phase of mitosis. NIH/3T3 cells were synchronized as described under “Experimental Procedures.” The cells were synchronized by serum starvation and then stimulated to enter cell cycle through serum stimulation. *FGF1* was not detected in serum-starved cells (0 h), nor at 2 h of serum addition (Fig. 7, A and B). However, at 16 h after serum addition, *FGF1* was detected specifically during the cytokinesis phase of mitosis (Fig. 7C), with as high as 98.3% cells expressing FGF1 (Table I). This trend continued up to 24 h after serum addition (Fig. 7D), although the percentage of *FGF1* positive cells decreased slightly (90.5%).

**Table I.** Percentage of *FGF-1* positive cells in cell cycle

| Time (h) | FGF-1 positive | FGF-1 negative | Total mitotic cells | FGF-1 positive % |
|----------|----------------|----------------|---------------------|------------------|
| 16       | 57             | 1              | 58                  | 98.3             |
| 24       | 38             | 4              | 42                  | 90.5             |

* N/A, not applicable.
CARG site target of de novo protein synthesis-independent cascade. This cascade does not involve Mek1 nor p38 MAP kinase. This cascade may have particular biological relevance in protection of tissue via acute ischemic preconditioning (31). Thus, temporal expression of FGF1 upon brief periods of hypoxia may provide a protective effect from detrimental effects of prolonged hypoxia, consistent with the role of FGF1 as a survival factor.

Our results also demonstrate regulation of the FGF1 gene by the Ets family of transcription factors. Indeed the FGF1 promoter D constructs with Ets mutations have low level basal activities. As shown in Fig. 4B, the percentage of induction for the Ets mutant by Rac1 and Cdc42 is 43 and 114%, respectively. These figures are not high in comparison to those for the wild type promoter, 262 and 488%, and for the CARG mutant, 248 and 303%, respectively. These results clearly indicate that the Ets element plays a significant role in Rac1 and Cdc42 induction. This observation is also reflected in Fig. 6C, demonstrating synergy between Ets and GTPases. In contrast, Ras manifests a less dramatic effect than Rac1 and Cdc42 (Fig. 5B, also Fig. 6A). These observations are in agreement with previous reports describing phosphorylation of Thr72 of Ets2 by Ras, which is necessary for increasing the trans-activation potential of this transcription factor (21). That this effect was not because of overexpression in our transient assays was demonstrated by selective activation by Ets2 and not PEA3. Together, these results directly link FGF1 function to the established role of Ets in development and angiogenesis (32).

Ras superfamily GTPases regulate growth, differentiation, as well as link cell surface receptors to organization of the actin cytoskeleton. These GTPases regulate actin dynamics and regulate fundamental processes such as cell movement, cell cycle progression, cytokinesis, as well as gene expression in the nucleus (29, 30). Rac1 and Cdc42 are wound-activated and are linked to actin-polymerized structures lamellipodia and filopodia formation, respectively. Rac1 is essential for producing leading edge protrusions, necessary for forward movement in the wound; Cdc42 is important for maintaining the polarized phenotype of migrating cells, whereas Ras is involved in stress fiber formation and focal adhesion turnover and also is required for cell movement. Altogether, it is the coordinated role of these small GTPases that leads to cell movement. Combined with the data presented in this study, FGF1 is likely involved in this fundamental process.

Not all GTPases regulate FGF1, and this observation further eliminates nonspecific effects because of overexpression. For example, RhoA showed no effect on FGF1 nor FGF2 regulation. The transient transfection data were supported by endogenous FGF1 expression in dividing cells (Fig. 7). These observations suggest that intracrine/autocrine signals involved in cell division require FGF1. Because cell migration, cell cycle progression, and cytokinesis involve actin reorganization and GTPases, we suggest that FGF1 is indeed necessary in these fundamental processes.

Acknowledgments—We thank Shunhua Xing for technical assistance; M. Ostrowski for helpful discussions and Ets-2 and PEA3 expression plasmids; R. Treisman for activated RhoAV14, Rac1-V12, and Cdc42-V12 expression plasmids; A. Hall for dominant inhibitory Rac1 V12N17 expression plasmid; D. Bar-Sagi for RasV12, RasV12S35, and RasV12C40 expression plasmids; R. Florkiewicz for FGF2 chlor-amphenol acetyltransferase reporter construct; A. Thoburn for the c-fos promoter-driven luciferase reporter.

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