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Exogenously Added Fibroblast Growth Factor 2 (FGF-2) to NIH3T3 Cells Interacts with Nuclear Ribosomal S6 Kinase 2 (RSK2) in a Cell Cycle-dependent Manner*[§]

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Fibroblast growth factor 2 (FGF-2) has been detected in the nuclei of many tissues and cell lines. Here we demonstrate that FGF-2 added exogenously to NIH3T3 cells enters the nucleus and interacts with the nuclear active 90-kDa ribosomal S6 kinase 2 (RSK2) in a cell cycle-dependent manner. By using purified proteins, FGF-2 is shown to directly interact through two separate domains with two RSK2 domains on both sides of the hydrophobic motif, namely the NH₂-terminal kinase domain (residues 360–381) by amino acid Ser-117 and the COOH-terminal kinase domain (residues 388–400) by amino acids Leu-127 and Lys-128. Moreover, this interaction leads to maintenance of the sustained activation of RSK2 in G₁ phase of the cell cycle. FGF-2 mutants (FGF-2 S117A, FGF-2 L127A, and FGF-2 K128A) that fail to interact *in vitro* with RSK2 fail to maintain a sustained RSK2 activity *in vivo*.

Fibroblast growth factor 2 (FGF-2)¹ is a member of the large FGF family consisting of 30 members in humans (1). It is involved in various cellular processes such as stimulation of DNA synthesis and cell proliferation, as well as differentiation and cell migration. *In vitro*, numerous cell types synthesize FGF-2 in five molecular isoforms. Four of these isoforms have high molecular masses (21.5, 22, 24, and 34 kDa) and are initiated at alternative CUG codons. The main form (18 kDa) is initiated at a regular AUG codon. The high molecular mass forms localize exclusively in the nucleus, their NH₂-terminal extension containing a nuclear localization sequence (2, 3). The 18-kDa isoform is primarily cytoplasmic and, despite the lack of a classical signal peptide, is released by a mechanism bypassing the classical export route taken by

most secreted proteins via the endoplasmic reticulum and the Golgi apparatus (4).

The pleiotropic effects exhibited by 18-kDa FGF-2 reflect an intricate combinatorial process involving interactions between the growth factor, any of four closely related high affinity transmembrane tyrosine kinase receptors (FGFR1–4), and low affinity binding sites corresponding to the naturally heterogeneous glycosaminoglycan chains of heparan sulfate proteoglycans. The interaction of FGF-2 with its receptor induces a phosphorylation cascade that results in the activation of signalization pathways. Furthermore, in addition to interactions with cell surface receptors, several growth factors enter the nucleus of target cells, either alone or associated with their receptors (5–9). Nuclear translocation of internalized FGF-1 and FGF-2 is an essential step in their mitogenic activity (10–12). FGF-2 signaling through both FGF receptors and nuclear targets is required for the stimulation of cell proliferation (13, 14). These data show that nuclear localization is a general phenomenon for some growth factors, suggesting nuclear functions independent of the functions as extracellular factors.

For the understanding of the nuclear functions of FGF-2, identification of interacting proteins is a crucial step. Here, we report that the exogenously added FGF-2 transiently interacts with nuclear active protein kinase RSK2. Using purified proteins, we show that FGF-2 interacts directly through two separate domains with two RSK2 domains. This interaction is cell cycle-dependent and furthermore maintains the RSK2 activity in NIH3T3 cells undergoing G₀/S transition. The interaction of nuclear FGF-2 with pluripotent RSK2 offers a new mechanism through which FGF-2 may control fundamental cellular processes.

MATERIALS AND METHODS

Reagents and Antibodies—All protein kinase inhibitors used were from Calbiochem. The monoclonal anti-RSK antibody was provided by BD Transduction Laboratories. The polyclonal anti-RSK1, anti-RSK2, phospho-specific antibodies, anti-phospho-histone H3 antibodies, and purified RSK2 were from Upstate Biotechnology. The monoclonal 1C1 anti-phospho-Ser-227 (NH₂ terminus RSK2 domain) monoclonal antibody was a gift of P. Sassone-Corsi (Institut de Genetique et de Biologie et Cellulaire, Strasbourg, France). The polyclonal anti-histone H3 antibody was a gift of D. Trouche (Institut de Biologie Cellulaire et Génétique, Toulouse, France). Anti-HA monoclonal and anti-FGF-2 polyclonal antibodies were provided by Babco and Chemicon, respectively. Control mouse IgGs were from Sigma. Histones H1 and H3 were from Roche Diagnostics. M-280 streptavidin beads were provided by Dynal. The HA-tagged FGF-2 expressing vector was described previously (15).

Plasmid Constructs—NH₂-terminally HA-tagged mouse RSK2 and HA-RSK2 deletion mutants cloned in pMT2 were provided by Morten Frödin (Department of Clinical Biochemistry, Glostrup Hospital, Glostrup, Denmark). The human FGF-2 cDNA was subcloned into the

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[§] The on-line version of this article (available at www.jbc.org) contains supplemental Fig. S1 (“FGF-2 S1127A does not interact with RSK2 (1–389 NTK), and FGF-2 L127A and K128A destabilize the interaction with RSK2 (–HM CTK)”).

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¹ The abbreviations used are: FGF-2, fibroblast growth factor 2; B-FGF-2, biotinylated FGF-2; CK2, casein kinase 2; CTK, carboxyl-terminal kinase; FGFR, FGF receptor; FCS, fetal calf serum; HA, hemagglutinin; HM, hydrophobic motif; MAP, mitogen-activated protein; MOPS, 4-morpholinepropanesulfonic acid; MSK, mitogen and stress-activated protein kinase; NTK, NH₂-terminal kinase; RSK, 90-kDa ribosomal S6 protein kinase; WT, wild type.

SmaI/XbaI sites of pGEX-KG. The XbaI-SmaI fragment FGF-2-(Δ 115–129), FGF-2-(Δ 115–119), and FGF-2-(Δ 124–129) cDNAs were prepared by PCR mutagenesis of the parental plasmid pGEX-KG. The glutathione *S*-transferase fusion FGFs were expressed and purified as described by the manufacturer's instructions (Amersham Biosciences), except that they were further purified using a heparin-Sepharose CL6-B column (Amersham Biosciences).

Cell Culture and Nuclear Fractionation—NIH3T3 cells were grown in Dulbecco's modified Eagle's medium with 10% fetal calf serum (FCS). To obtain G_0 -arrested cells, cells were preincubated for 48 h in Dulbecco's modified Eagle's medium with 0.5% FCS. The cells were then stimulated with FGF-2 (20 ng/ml medium) to enter the G_1 phase. At different times after the stimulation, cells were harvested by trypsinization and centrifuged at $600 \times g$ for 10 min at 4 °C in Dulbecco's modified Eagle's medium with 10% FCS. Nuclei and post-nuclear supernatants (cytoplasm) were prepared as described previously (16) in Buffer A containing 15 mM Tris-HCl, pH 7.5, 0.15 M NaCl, 5 mM $MgCl_2$, 0.1% Tween 20, and a mixture of protease and phosphatase inhibitors (Roche Applied Science and Calbiochem respectively). Nuclear extracts were carried out according to Bailly *et al.* (13).

COS7 cells were cultured and transfected using Lipofectamine 2000 (Invitrogen Inc.) as described previously (17). After transfection, cells were cultured for 48 h and without serum during the final 24 h. Cells were harvested as described above and resuspended in Buffer A without NaCl for 30 min on ice. Then the NaCl concentration of Buffer A was adjusted to 0.4 M, and lysed cells were extracted for 30 min at 4 °C in a rotating wheel. Cell extracts were clarified by centrifugation ($14,000 \times g$ for 10 min) at 4 °C.

Immunoprecipitation and Western Blotting—Nuclear extract and cytoplasm corresponding to 10^7 NIH3T3 cells were used in each immunoprecipitation assay carried out in Buffer A with 0.4 M NaCl. The subcellular fractions were preincubated at 4 °C for 1 h in a rotating wheel with 20 μ l of protein G-Sepharose beads (Amersham Biosciences). Protein G-Sepharose beads were precipitated by centrifugation, and the supernatants were incubated overnight at 4 °C with 4 μ g of each antibody or control IgG. The immunocomplexes were then captured with 10 μ l of 50% protein G-Sepharose at 4 °C for 1 h. Sepharose bead immunocomplexes were precipitated by centrifugation, washed five times with Buffer A and 0.4 M NaCl through a cushion of 1 M sucrose in Buffer A with 0.4 M NaCl and dissolved in 1 \times Laemmli sample buffer. For kinase assays, the last two washes were done with kinase assay buffer.

Western blotting was performed as described previously (18). Clarified cellular extracts from COS7 expressing full-length or RSK2 deletion mutants were incubated for 1 h at 4 °C in the presence of FGF-2 wild type or FGF-2 deletion mutants in Buffer A with 0.4 M NaCl. Anti-HA antibody was then added, and the incubation was prolonged for 90 min with the addition of 10 μ l of 50% protein G-agarose beads during the final 30 min. Sepharose bead immunocomplexes were processed as described above.

Biotinylated FGF-2 Interaction with Nuclear Extract Proteins—Biotinylated FGF-2 (B-FGF-2)-loaded beads (5 μ l), prepared as described previously (18), were incubated for 1 h at 4 °C in 0.3 ml of Buffer A containing 2 μ g of proteins from nuclear extract. After extensive washing, bound proteins were eluted at 4 °C with 50 μ l of elution buffer (Buffer A with 0.4 or 0.5 M NaCl). Eluates were used for immunoprecipitation and/or tested for kinase assays.

Kinase Assays—Aliquots of the 0.5 M NaCl eluates (2 μ l) or immunocomplexes were incubated for 15 min at 30 °C with 50 μ M ATP (10 μ Ci of [γ - 32 P]ATP) (3000 dpm/pmol), 0.1 μ g of histone H3 (Roche Applied Science) in a final volume of 10 μ l of 20 mM MOPS, pH 7.2, 25 mM β -glycerol phosphate, 5 mM EGTA, 5 mM $MgCl_2$, 1 mM dithiothreitol, and 1 mM sodium orthovanadate. Reactions were stopped by adding 5 μ l of 5 \times Laemmli sample buffer, and the samples were analyzed by SDS-PAGE, transferred onto nitrocellulose or polyvinylidene difluoride membrane, and autoradiographed and/or immunoblotted.

RESULTS

FGF-2 Interacts *In Vitro* with RSK2—We have reported previously that the activation of resting cells by FGF-2 resulted in the phosphorylation of a subset of nuclear proteins, among which is H3 histone (19). Several of the newly phosphorylated proteins are known to be casein kinase 2 (CK2) substrates (19, 20). In agreement with this result, we have shown a direct interaction between CK2 and nuclear FGF-2 *in vitro* by incubation of a nuclear extract with B-FGF-2 immobilized on

streptavidin beads (18) and also *in vivo* by coimmunoprecipitation of CK2 and FGF-2 from NIH3T3 cells undergoing G_0/S transition (13). However, H3 histone is not a CK2 substrate.

To identify the kinase activities able to interact with B-FGF-2 and phosphorylate H3, we incubated a nuclear extract from NIH3T3 exponential growing cells with B-FGF2 immobilized on streptavidin beads and eluted bound proteins at 0.4 and 0.5 M NaCl and performed a kinase assay. Kinase activity/activities were detected among the proteins bound to B-FGF-2 as well as in 0.4 and 0.5 M NaCl fractions with or without the addition of histone H3 as an exogenous substrate (Fig. 1A). In this assay, as described previously (18) CK2 was eluted at 0.4 M NaCl, and the 0.5 M fraction was unable to phosphorylate exogenously added casein (data not shown). To further characterize the kinase activity/activities from the 0.5 M fraction we used H3 as substrate and two inhibitors of cyclin-dependent kinases, olomoucine and roscovitine, as well as staurosporine, a potent and broad spectrum inhibitor of kinases (21). None of these inhibitors altered the levels of H3 phosphorylation (Fig. 1B, *top*). Next, we used Ro318220, an inhibitor of the protein kinase C isoforms as well as RSK1, RSK2, and MSK1 (22, 23), and found that Ro318220 blocked the protein kinase activity/activities in a dose-dependent manner (Fig. 1B, *bottom left*). Because the protein kinases C are also sensitive to staurosporine, we deduced that the kinase activity/activities of the 0.5 M fraction could correspond to RSK1 and/or RSK2 and/or MSK1 kinases. However, H-89, an inhibitor of MSK1 (24), did not affect the kinase activity/activities of the 0.5 M fraction (Fig. 1B, *bottom right*). Furthermore, although RSK1 and RSK2 were detected in the nuclear extract (Fig. 1C, *top left*), only RSK2 bound to FGF-2 and was present in 0.4 and 0.5 M fractions (Fig. 1C, *top right*). Next, we sought to determine whether RSK2 is in its active form in 0.4 and 0.5 M fractions. The phosphorylation of serine 227 in the NH_2 -terminal domain of RSK2 is the real indicator of RSK2 activity. We therefore used a monoclonal antibody directed against phosphoserine 227 in a similar experiment. As shown in Fig. 1C, *bottom right*, RSK2 bound to FGF-2 and was present in 0.4 and 0.5 M fractions in its active form.

RSK2 has been shown to phosphorylate histone H3 on the serine 10 in epidermal growth factor-stimulated human fibroblasts (25). To determine whether the FGF-2 bound RSK2, in which Ser-227 is phosphorylated, is able to phosphorylate histone H3 on Ser-10, we immunoprecipitated RSK2 from the 0.5 M fraction and performed a kinase assay with histone H3 as a substrate. As shown in Fig. 1D, histone H3 was specifically phosphorylated on Ser-10 by immunocomplexes obtained with anti-RSK and anti-RSK2 antibodies, respectively. In control experiments using unrelated IgG, we did not detect kinase activity toward histone H3 phosphorylation (Fig. 1D, *left lane*). In addition, RSK1 activity was not detected in the 0.5 M fraction (Fig. 1D, *lane marked Anti-RSK1*). We concluded that RSK2 molecules bound to FGF-2 are able to phosphorylate Ser-10 of histone H3.

To ascertain that the FGF-2/RSK2 interaction was direct and specific, we incubated immobilized B-FGF-2 with purified RSK2 (26). As shown in Fig. 1E, RSK2 bound directly to FGF-2 (*lane 2*). Furthermore, the addition of an excess of unbiotinylated FGF-2 completely prevented the interaction of RSK2 with B-FGF-2 (Fig. 1E, *lanes 3 and 4*), whereas the addition of cytochrome *c*, a protein similar in molecular mass and isoelectric point to FGF-2, had no effect (Fig. 1E, *lanes 5 and 6*). Taken together, these data demonstrate a direct *in vitro* interaction between FGF-2 and RSK2.

Two Domains of FGF-2 Interact with Two Domains of RSK2—Like the other four members of its family, RSK2 has

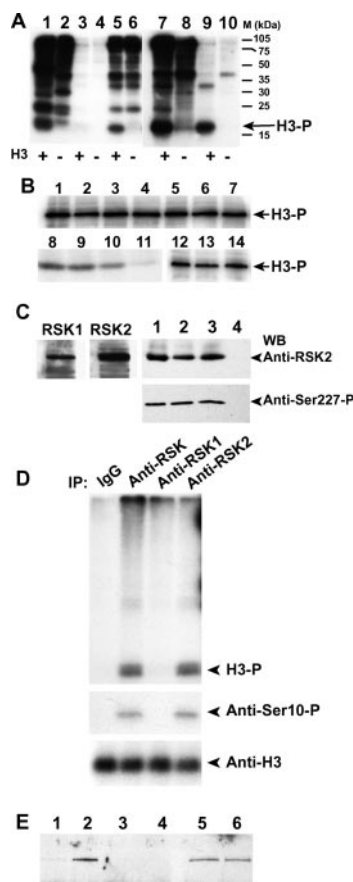


FIG. 1. FGF-2 interacts directly with active RSK2. **A**, a nuclear extract (2 μ g) from growing NIH3T3 cells was incubated with biotinylated FGF-2 (B-FGF-2) loaded on streptavidin beads (5 μ l) or with unloaded beads (5 μ l). Bound proteins were eluted with 50 μ l of 0.4 and 0.5 M NaCl successively. An aliquot of nuclear extract (0.1 μ g) (lanes 1 and 2), unloaded beads (lanes 3 and 4), FGF-2-bound proteins (lanes 5 and 6), and 0.4 and 0.5 M NaCl fractions (lanes 7–10, respectively) were incubated in the kinase assay buffer with (+) or without (–) histone H3 (H3-P) as an exogenous substrate. Proteins were resolved by SDS-PAGE, electrotransferred to nitrocellulose, and autoradiographed. Molecular mass markers are shown on the right in kilodaltons. **B**, FGF-2 associates with Ro 318220-sensitive and H89-insensitive protein kinase activity. Aliquots of the 0.5 M NaCl fraction were incubated in the kinase assay buffer with 0.1 μ g of H3 (H3-P) as substrate without (lanes 1, 8, and 12) or with 25 and 250 μ M olomoucine (lanes 2 and 3, respectively), 10 and 100 nM staurosporine (lanes 4 and 5, respectively), 25 and 100 nM staurosporine (lanes 6 and 7), 0.05, 0.5, and 5 μ M Ro 318220 (lanes 9–11 respectively), and 0.1 and 10 μ M H89 (lanes 13–14, respectively) inhibitors on the kinase activity contained in the 0.5 M NaCl fraction. Kinase assay products were processed as for panel A. **C**, immunodetection of RSK2 among the FGF-2 bound proteins. *Left*, immunoblot of nuclear extract (0.1 μ g) probed with antibodies raised against RSK1 and RSK2. *Right*, immunoblot using anti-RSK2 (top) or anti-Ser-227 antibody (Anti-Ser227-P; bottom); lane 1, proteins bound to FGF-2-loaded beads; lanes 2 and 3, 0.4 and 0.5 M NaCl fraction, respectively; lane 4, unloaded beads. **WB**, Western blot. **D**, 0.5 M fraction contains active RSK2. Immunoprecipitation (IP) from 0.5 M fraction (10 μ l) with 0.1 μ g of IgG control or anti-RSK, anti-RSK1, and anti-RSK2 antibodies. Kinase assays with immunocomplexes as the kinase supply were carried out, and kinase assay products were processed as for panel A. *Top*, autoradiography (H3-P); *middle and bottom*, immunoblot using the anti-phospho-Ser10-histone H3 (Anti-Ser10-P; *middle*) and the anti-histone H3 (Anti-H3). **E**, RSK2 interacts directly with FGF-2. Streptavidin beads were first loaded with an excess of B-FGF-2. Streptavidin beads were washed extensively. These beads were incubated with purified recombinant RSK2 with or without large amounts FGF-2 or cytochrome *c*. After 1 h at 4 $^{\circ}$ C, unbound proteins were removed by extensive washing, and the bound complexes were processed for immunoblotting with an anti-RSK2 polyclonal antibody. Lane 1, unloaded beads; lanes 2–6; loaded beads; lane 2, without competitor; lanes 3 and 4, with 1 and 5 μ g of native FGF-2 as a competitor, respectively; lanes 5 and 6, with 1 and 5 μ g of cytochrome *c* as a competitor, respectively.

two kinase domains (NTK and CTK domains) connected by a regulatory linker region (Fig. 2A, top). To map the FGF-2 interacting domain in RSK2, immobilized B-FGF-2 was incubated with cellular extracts of COS7 expressing HA-tagged full-length RSK2 or deletion mutants (Fig. 2A, middle). FGF-2 interacts with RSK2-(1–389)-NTK, encompassing the hydrophobic motif (HM) (1–389 NTK), and with RSK2-(388–740)-CTK, excluding the HM (–HM CTK). The RSK2-(1–360)-NTK and RSK2-(401–740)-CTK mutants do not interact with B-FGF-2 (Fig. 2A, bottom). This finding suggests that the RSK2 sequences between residues 361–381 and residues 388–400 correspond to the domains interacting with the FGF-2. It is noteworthy that the sequence between the residues 390–400 is highly divergent, and the sequence between the residues 361–381 is conserved in the RSK and MSK families (Fig. 2D, bottom). These two FGF-2-recognized RSK2 domains are not homologous to each other. We thus hypothesize that FGF-2 interacts with RSK2 through two different domains.

To delineate the FGF-2 domains interacting with RSK2, we constructed a series of FGF-2 deletion mutants. Their ability to bind RSK2, RSK2-(1–389)-NTK, and RSK2-(388–740)-CTK (–HM CTK) was determined by coimmunoprecipitation (Fig. 2B). We used the FGF-2-(131), in which the last 24 residues in the carboxyl end of the molecule were deleted, and the FGF-2-(Δ 115–129), which lacks the residues 115–129. The FGF-2-(131) interacts with RSK2 as well as the FGF-2 wild type does, in contrast to the FGF-2-(Δ 115–129) mutant, which interacts neither with the full-length RSK2 (Fig. 2B, top) nor with the 1–389 NTK or –HM CTK RSK2 mutants (Fig. 2B, bottom). To better characterize the RSK2 interaction domain of FGF-2, we constructed two FGF-2 mutants, FGF-2-(Δ 115–119) and the FGF-2-(Δ 124–129), lacking, respectively, the residues 115–119 and 124–129. FGF-2-(Δ 115–119) fails to interact with RSK2-(1–389)-NTK, and neither does FGF-2-(Δ 124–129) interact with RSK2-(388–740)-CTK (–HM CTK). In addition, like FGF-2-(Δ 115–129), FGF-2-(Δ 115–119) and FGF-2-(Δ 124–129) fail to interact with full-length RSK2 (Fig. 2B, top).

Next, we wished to identify amino acids in these two FGF-2 domains that could account for recognition of the two FGF-2-recognized RSK2 domains. With this aim in view, we constructed several FGF-2 proteins with single point mutation in the two RSK2 interacting domains and tested their capacity to coimmunoprecipitate with HA-RSK2. As shown in Fig. 2C, the substitution of serine 117, leucine 127, or lysine 128 by alanine (S117A, L127A, and K128A respectively) is sufficient to lose the interaction with full-length RSK2 (supplemental Fig. S1, available in the on-line version of this article).

We conclude that FGF-2 interacts with RSK2 through two separate domains, and our results suggest that these two domains together are required to maintain the interaction with full-length RSK2. FGF-2 interacts through the serine 117 with the NTK of RSK2 at the site delineated by the residues 361–381 before the HM, through the leucine 127 and the lysine 128 with the CTK at the site delineated by the residues 388–400 after the HM (Fig. 2D).

FGF-2-RSK2 Complexes Were Detected in Vivo, and FGF-2/RSK2 Interaction Is Cell Cycle-dependent—We then sought to determine the existence of FGF-2-RSK2 complexes *in vivo*. First, using streptavidin beads, we isolated these complexes from nuclear and cytoplasm extracts of asynchronous growing NIH3T3 cells that underwent a 4 h stimulation by B-FGF-2 (Fig. 3A, top). RSK2 was found to be associated to B-FGF-2 only in the nuclear extract from stimulated cells. However, B-FGF-2 accumulated both in the nucleus and in the cytoplasm (Fig. 3A, bottom). To confirm the existence of FGF-2-RSK2 complexes in an independent experiment, asynchronous NIH3T3 cells were

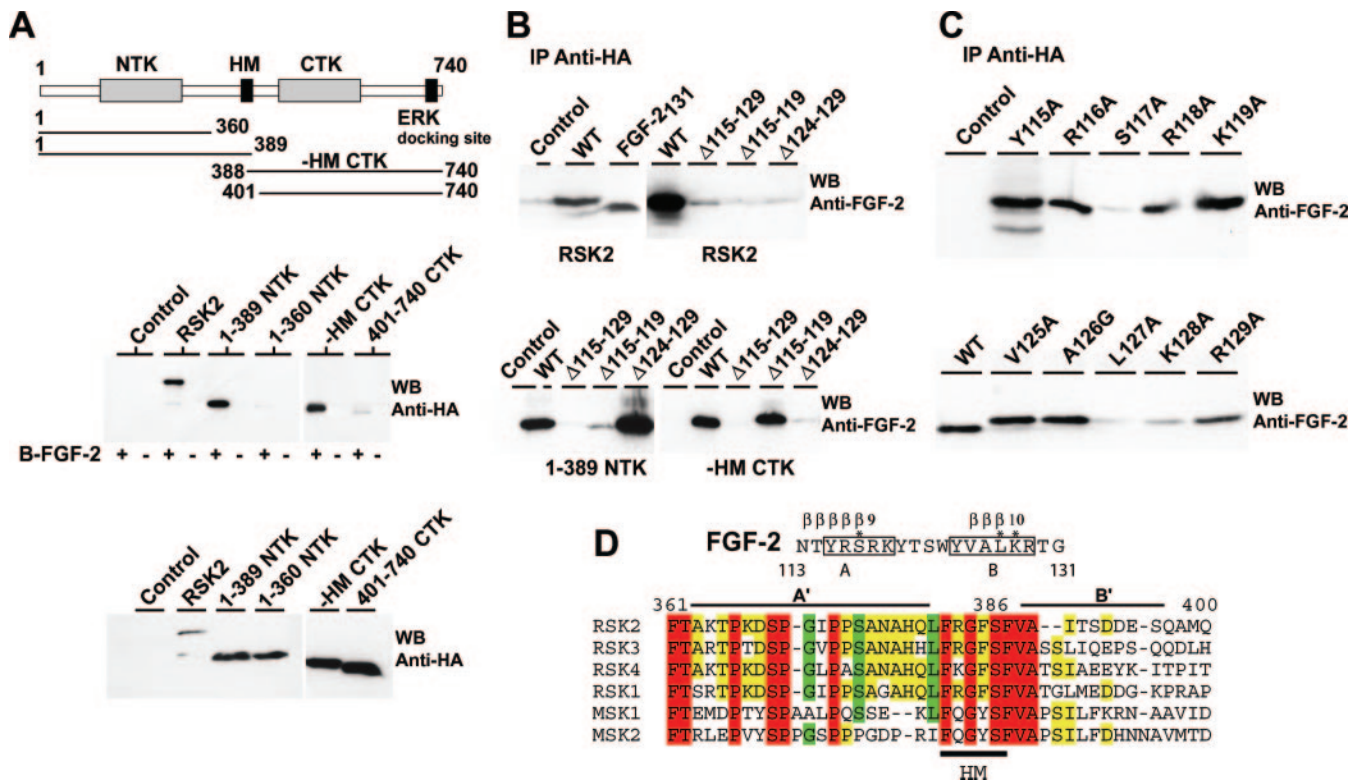


FIG. 2. Two FGF-2 separate domains interact with two RSK2 separate domains. *A*, *top*, structure of RSK2. RSK2 is composed of two kinase domains, NTK and CTX, connected by a regulatory linker region. The carboxyl-terminal tail contains a docking site for extracellular signal-regulated kinase (ERK), and the linker contains the HM. *Arrows* below the scheme indicate the position and the length of RSK2-deletion mutants (mouse RSK2 numbering). *Middle*, FGF-2 interacts with two RSK2 domains. B-FGF-2-loaded streptavidin beads (+) or unloaded beads (–) were incubated with cellular extract of COS7 cells expressing the empty vector as control or with cellular extract of COS7 cells expressing HA-tagged full-length RSK2 or deletion mutants. The bound complexes were immunoblotted with an anti-HA monoclonal antibody. *Bottom*, before the interaction, assay aliquots were processed as described above with an anti-HA monoclonal antibody. *WB*, Western blot. *B*, RSK2 interacts with two FGF-2 separate domains. Wild type (WT) or FGF-2 deletion mutants (10 pmol) were incubated with cellular extract of COS7 cells expressing HA-RSK2 (*top*) or HA-RSK2 deletion mutants (*bottom*; *left*, HA-(1–389)-NTK; *right*, –HM CTX). Immunoprecipitations (IP) were carried out as described under “Materials and Methods,” and immunocomplexes were immunoblotted with an anti-FGF-2 polyclonal antibody. *Control* corresponds to immunoprecipitation performed with unrelated IgG. *C*, FGF-2 recognizes RSK2 through three amino acids, serine 117, leucine 127, and lysine 128. Wild type (WT) or FGF-2 deletion mutants (10 pmol) were incubated with cellular extract of COS7 cells expressing HA-RSK2, and immunoprecipitations (IP) were processed as for *panel B*. *WB*, Western blot. *D*, *top*, FGF-2 sequence between the residues 113–131. *A* and *B* correspond to the two separate domains interacting with RSK2. The location and the length of the β -strands are shown above the sequence. The secondary structure assignment is in accordance with the published nomenclature, with the β -strands labeled from 1 through 12. The deletion mutants used to map the RSK2-recognized FGF-2 domains are boxed, and the FGF-2 residues interacting with RSK2 are denoted by asterisks. *Bottom*, amino acid alignment of RSK and MSK kinases encompassing FGF-2 interacting domains *A'* and *B'* on both sides of the HM, recognized by FGF-2 domains *A* and *B*, respectively.

cultured in the presence of HA-FGF-2 for 4 h, and FGF-2-RSK2 complexes were immunoprecipitated by anti-RSK2 antibodies or unrelated IgG as control (Fig. 3*B*). FGF-2-RSK2 complexes were only detected in nuclear extracts immunoblotted with anti-HA and never in control experiments. Thus, isolation of the complexes with FGF-2 or RSK2 antibodies resulted in the same data, reinforcing the existence of FGF-2-RSK2 complexes *in vivo* in asynchronous growing cells. Modified FGF-2 (B-FGF-2) and tagged FGF-2 (HA-FGF-2) used in these experiments have the same mitogenic activities as wild type FGF-2 (13, 15). Second, to examine whether the RSK2 associated with FGF-2 was in an active form, we incubated the complexes isolated in Fig. 3*A* in a kinase assay with H3 as an exogenous substrate. Complexes isolated from nuclear extracts phosphorylated H3 as well as several other proteins (Fig. 3*C*, *lane 1*). A weak kinase activity was detected in cytoplasm (Fig. 3*C*, *lane 2*), and no activity was found in unstimulated cells (Fig. 3*C*, *lanes 3* and 4).

Moreover, we searched for FGF-2-RSK2 complexes in asynchronous NIH3T3 grown in the presence of FGF-2 mutants that do not interact with RSK2 *in vitro*. As shown in Fig. 3*D*, no FGF-2-RSK2 complexes were detected in FGF-2 mutant-stimulated cells. Yet, just as wild type FGF-2 does, S117A, L127A,

and K128A FGF-2 mutants accumulate in the cytoplasm and the nucleus of the cells (Fig. 3*E*).

Having established the existence of FGF-2-RSK2 complexes essentially in the nucleus of asynchronous growing cells, we postulated that this interaction could be cell cycle-dependent because cellular FGF-2 uptake occurred continuously through the cell cycle, whereas FGF-2 entered the nucleus of target cells during the G_1 phase (16). Quiescent (G_0) NIH3T3 cells were triggered to re-enter the cell cycle by the addition of HA-FGF-2. The transition between early and late G_1 occurred 10 h after stimulation (27), and NIH3T3 cells entered the S phase after 12 h (13). We have focused our study on two periods of time after FGF-2 addition, namely during early G_1 (2–4 h) after the first transient activation of the MAP kinase signaling pathway, and at the vicinity of the restriction point in late G_1 (10 h) (27).

Immunoprecipitations with anti-RSK2 antibodies were carried out at these different time points after the stimulation of cells. RSK2 was not detected in nuclear extracts of G_0 -arrested cells (Fig. 3*F*, *section N*, *top*). FGF-2-RSK2 complexes were detected in nuclear extracts in early G_1 (2–4 h) with a maximum at 4 h after stimulation of the cells and not in late G_1 (Fig. 3*F*, *section N*, *top*). Yet, the amounts of nuclear HA-FGF-2 during early and late G_1 were not significantly different (Fig.

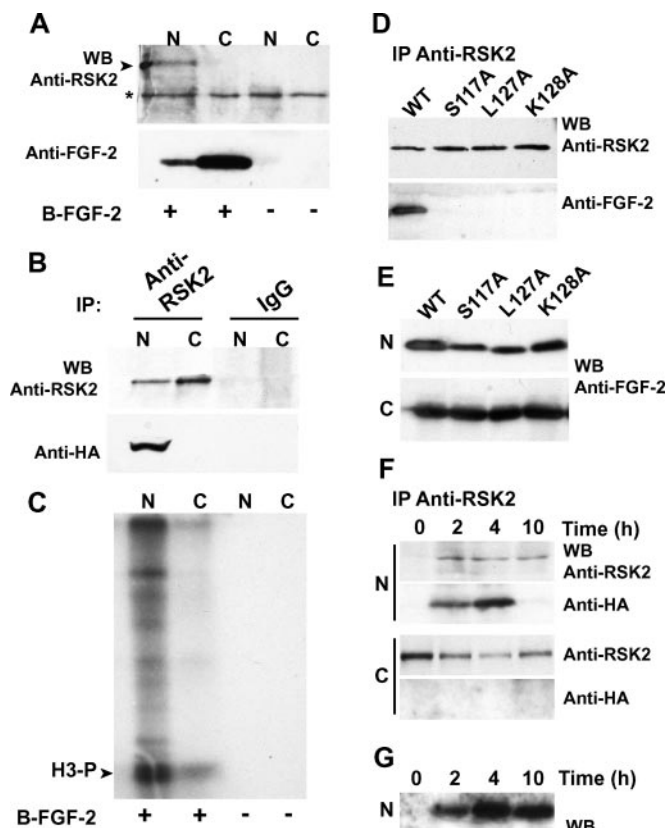


FIG. 3. FGF-2 interacts *in vivo* with nuclear RSK2. Asynchronous NIH3T3 cells were grown in the presence (+) or absence (–) of B-FGF-2 (A) or HA-tagged FGF-2 (20 ng/ml) (B) for 4 h. A, cells were fractionated, and the nuclear extracts (lanes marked N) and cytoplasm (lanes marked C) were incubated with streptavidin beads (5 μ l). Bound proteins were processed for immunoblotting. Top, immunoblot with anti-RSK2 polyclonal antibody (asterisk, nonspecific). Bottom, immunoblotting with anti-FGF-2 monoclonal antibody. WB, Western blot. B, nuclear extracts (N) and cytoplasm (C) were immunoprecipitated (IP) with anti-RSK2 or IgG control, and immunocomplexes were immunoblotted with the anti-RSK2 (top) or anti-HA monoclonal antibodies (bottom). WB, Western blot. C, RSK2 bound to FGF-2 phosphorylates histone H3. Bound proteins obtained as for panel A were incubated in a kinase assay with histone H3 (0.1 μ g), and the kinase assay products were processed for autoradiography; lanes N and C are as in panel A. D, asynchronous NIH3T3 cells were grown in the presence FGF-2 wild type (WT) or FGF-2 mutants (20 ng/ml) for 4 h. Cellular extracts were immunoprecipitated (IP) with anti-RSK2, and immunocomplexes were immunoblotted with anti-RSK2 (top) or anti-FGF-2 (bottom). WB, Western blot. E, FGF-2 cellular content. Cells were fractionated, and the nuclear extracts (N) and aliquot (one-tenth) of cytoplasm (C) were immunoblotted with anti-FGF-2 antibody. WT, wild type; WB, Western blot. F, cells (10^7) were harvested at different times after stimulation and processed as for panel B. Immunocomplexes from nuclear extracts (N) and cytoplasm (C) obtained with anti-RSK2, were immunoblotted with anti-RSK2 and anti-HA antibodies. IP, immunoprecipitation; WB, Western blot. G, HA-FGF-2 cellular content. The nuclear extracts (N) and aliquot (one-tenth) of cytoplasm (C) were immunoblotted with anti-HA antibody. WB, Western blot.

3G, section N). FGF-2-RSK2 complexes were never found in the cytoplasm (Fig. 3F, section C, bottom), whereas RSK2 and FGF-2 accumulated in this subcellular fraction (Fig. 3F, section C, bottom, and Fig. 3G, section C).

FGF-2 Interacts with Active Form of RSK2 and Maintains a High Level of the Kinase Activity *In Vivo*—We then tried to relate this cell cycle-dependent interaction to functional interpretation. We stimulated G_0 -arrested, HA-RSK2-transfected NIH3T3 cells by FGF-2. Immunoprecipitations of cellular extracts with anti-FGF-2 antibodies were carried out at different times after stimulation of the cells (Fig. 4A). FGF-2 was not

detected in cellular extracts of G_0 -arrested cells (Fig. 4A, top). FGF-2-RSK2 complexes were detected after 2 and 4 h of stimulation, and RSK2 associated with FGF-2 was phosphorylated on Ser-227 (Fig. 4A, middle and bottom, respectively). It is noteworthy that FGF-2-RSK2 complexes were not detected after 30 min of stimulation, whereas the same amount of FGF-2 was immunoprecipitated at different times after stimulation of the cells (Fig. 4A, top). Next, in another set of experiments we compared the ability of RSK2, which was immunoprecipitated from G_0 -arrested, HA-RSK2-transfected cells and then stimulated with FGF-2, FGF-2 K128A, or FCS, to phosphorylate H3 histone (Fig. 4B). The maximum of the kinase activity of RSK2 occurred 30 min after the stimulation of cells by FGF-2, FGF-2 K128A, or FCS. Only FGF-2 wild type maintained a sustained RSK2 activity (~80% of the maximum) in cells undergoing early G_1 (2–4 h). The FGF-2 K128A that does not interact with RSK2 is unable to maintain a high level of H3 phosphorylation. In addition, the number of RSK2 active molecules assessed by the phosphorylation level of Ser-227 is the same during the first 4 h of FGF-2 stimulation of the cells. On the contrary, when the cells are stimulated by FGF-2 K128A or FCS, Ser-227 is partly dephosphorylated within 2 h (Fig. 4C). However it is noteworthy that the number of RSK2-active molecules is the same during the first 30 min of stimulation of the cells by FGF-2, FGF-2 K128A, or FCS. This finding suggest that FGF-2 as well as FGF-2 mutants activate the FGF receptors, inducing a transient RSK2 activation. To study whether the mutants maintained the ability to activate the FGF receptors, we tested their effect on tyrosine phosphorylation of receptors. As shown in Fig. 4D, both wild type and mutated FGF-2 induced tyrosine phosphorylation of FGF receptors.

The sustained RSK2 activity induced by FGF-2 correlated with the level of Ser-10 phosphorylation of histone H3 in cells undergoing G_0 -S transition (Fig. 4E). In FGF-2 stimulated cells, Ser-10 remains phosphorylated in early G_1 (2–4 h), whereas this serine is only found phosphorylated 2 h after stimulation by FCS or FGF-2 K128A (Fig. 4E). The same results were obtained with cells stimulated with FGF-2 S117A or FGF-2 L127A (data not shown).

To determine the physiological relevance of the cell cycle dependent FGF-2/RSK2 interaction, we tested the mitogenic activities of FGF-2 mutants that fail to interact with RSK2. FGF-2 L127A or FGF-2 K128A have a mitogenic activity reduced by 50% and FGF-2 S117A by 80% as compared with that of wild type FGF-2 (Fig. 4F).

DISCUSSION

In this report, we identified RSK2 as a nuclear target of FGF-2. Exogenously added FGF-2 to G_0 -arrested NIH3T3 cells binds to nuclear RSK2 in a cell cycle-dependent manner. The FGF-2-RSK2 complexes in the nucleus of cells undergoing G_0 / G_1 transition contain the active form of RSK2. The important consequence of RSK2/FGF-2 interaction is the maintenance of RSK2 in active form when the cells were stimulated to grow by FGF-2.

In these complexes, FGF-2 recognizes two domains of RSK2 located on both sides of the HM. These two FGF-2-recognized RSK2 domains are divergent from each other. The domain located upstream of HM is conserved in the RSK and MSK families, but the one downstream of HM is highly divergent. FGF-2 interacts through the serine 117 with the NTK of RSK2 at the site delineated by the residues 361–381 before the HM, through the leucine 127 and the lysine 128 with the CTK at the site delineated by the residues 388–400 after the HM. It is noteworthy that a single mutation among these three residues abolishes the RSK2 interaction.

The three-dimensional structure of FGF-2 corresponds to

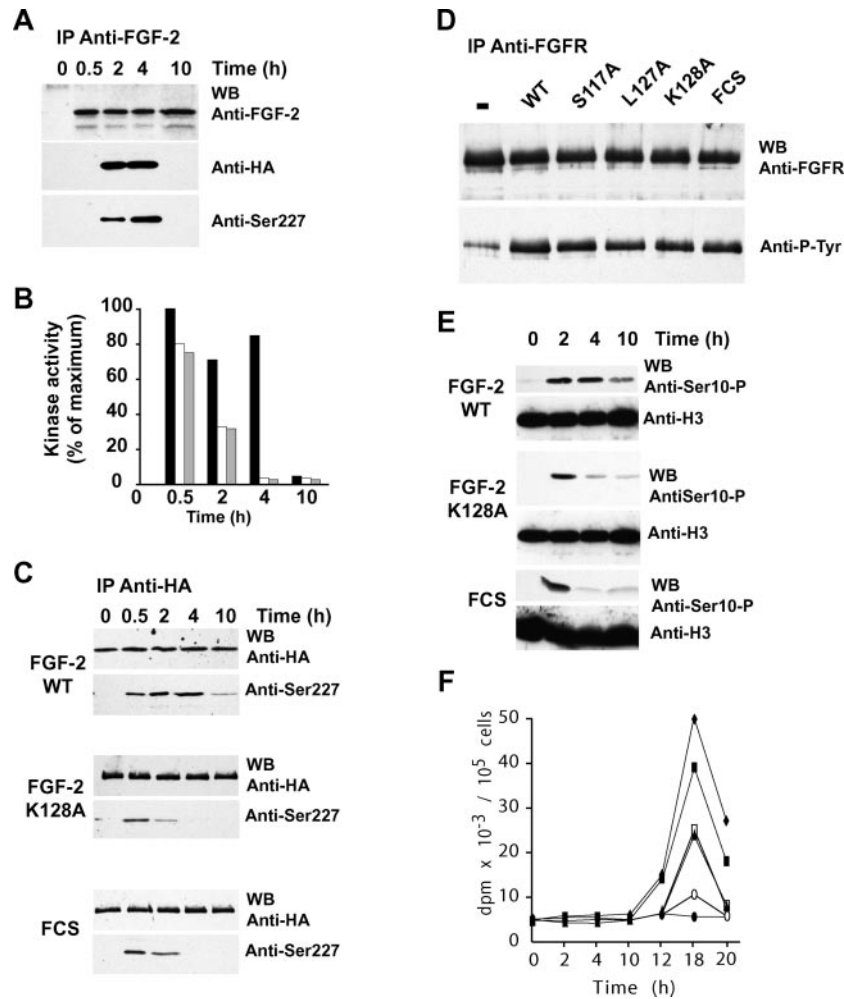


FIG. 4. Exogenously added FGF-2 induces a sustained RSK2 activity in cells undergoing early G₁. NIH3T3 cells were transfected with plasmids expressing HA-RSK2 (A, B, and C). After 48 h and a final 24 h of serum starvation, cells were stimulated with FGF-2 wild type (A) or with FGF-2 wild type, FGF-2 K128A, or FCS in the presence of H89 (10 μ M) (B and C). At different times after stimulation, cells extracts were processed for immunoprecipitation. A, immunoprecipitations (IP) were carried with anti-FGF-2 antibody. Immunocomplexes were immunoblotted with anti-FGF-2 (top), anti-HA (middle), and anti-Ser-227 antibodies (bottom). B, RSK2 was immunoprecipitated from cell extracts with anti-HA antibody, and the recovered kinase was assayed for its ability to phosphorylate H3 histone. Kinase assay products were processed for autoradiography and quantified by PhosphorImager scanning. Black bar (left), FGF-2; open bar (center), FGF-2 K128A; gray bar (right), FCS. C, immunocomplexes were immunoblotted with anti-HA (top) and anti-Ser-227 antibodies (bottom). IP, immunoprecipitation. D, autophosphorylation of FGFR1. G₀-arrested NIH3T3 cells were stimulated with FGF-2 wild type (WT) or mutated FGF-2 (20 ng/ml) for 5 min, harvested, and lysed. Immunoprecipitations (IP) were carried with anti-FGF receptor antibody. Immunocomplexes were immunoblotted with anti-FGF receptor (top) and with anti-phosphotyrosine (Anti-P-Tyr; 4G10) antibodies (bottom); dash above the far left lane denotes G₀-arrested cells. WT, wild type; WB, Western blot. E, phosphorylation of histone H3. Acid-soluble proteins were prepared by extracting nuclei with 0.4N H₂SO₄ (25) and processed for immunoblot with anti-phospho-Ser10-histone H3 (Anti-Ser10-P) and anti-H3 antibodies. WT, wild type; WB, Western blot. F, G₀-arrested NIH3T3 cells were stimulated with FGF-2 (20 ng/ml). Cells were pulse-labeled for 1 h at different times with 10 μ Ci of [³H]thymidine before harvesting, and the radioactivity incorporated was measured (3). \blacklozenge , FGF-2-stimulated cells; \blacksquare , FCS-stimulated cells; \circ , FGF-2 S117A-stimulated cells; \blacktriangle , FGF-2 L127A-stimulated cells; \square , FGF-2 K128A-stimulated cells; \bullet , G₀-arrested cells.

155 residues organized into 12 antiparallel β -strands connected by tight turns and loop regions arranged in three β -sheets composed of four β -strands (28). The Ser-117 residue corresponds to the carboxyl terminal residue of the ninth β -strand (¹¹³NTYRS¹¹⁷), and Leu-127 and Lys-128 corresponds to the two last residues of the 10th β -strand (¹²⁶ALK¹²⁸). The motif harboring the RSK2 interacting domain of FGF-2 (¹¹⁶RSRKYSWYVALKR¹²⁹) overlaps with the FGF-2 bipartite nuclear localization signal (¹¹⁶RSRK¹¹⁹ and ¹²⁸KR¹²⁹) and the binding site of CK2 (13, 29).

In vivo, the nuclear FGF-2-RSK2 complexes were isolated in a segment of G₁ (4 h) following the stimulation of the MAP kinase signaling pathway (27). The RSK2/FGF-2 interaction correlated perfectly with the maintenance of the high level of active RSK2 in cells stimulated by FGF-2 wild type. However, RSK2 reached a maximum of activity in cells stimulated by FGF-2 wild type as well as by FGF-2 K128A or FCS at the

beginning of the G₁ phase (30 min) during the transient stimulation of the MAP kinase signaling pathway (13, 27). FGF-2-RSK2 complexes were never detected at this time. Indeed, we propose that the externally added FGF-2 acts through a dual mode of signal transduction. First, FGF-2, as well as FGF-2 mutants, activates through cell surface receptors the MAP kinase signaling pathway that, in turn, activates RSK2. Secondly, FGF-2 as well as FGF-2 mutants accumulated in the nucleus, but only FGF-2 wild type, interacts with and maintains RSK2 in active form. At this time, we do not know if the interaction between FGF-2 and RSK2 results in an activation of RSK2 and/or in an increase of the half-life of RSK2 active form by inhibiting its deactivation. Indeed, FGF-2 mutants that fail to interact with RSK2 fail to maintain a sustained RSK2 activity. In line with this observation, the RSK2-interacting FGF-2 residues are not involved in binding to heparin and high affinity receptors, and the corresponding FGF-2 mu-

tants enter the nucleus as well as the FGF-2 wild type (13, 30, 31). Indeed, only the residues Lys-119 and Arg-129 in the FGF-2 nuclear localization signal are critical in regulating nuclear localization of the growth factor (29, 32). However, the FGF-2 mutants (S117A, L127A, and K128A) have reduced mitogenic activity.

In similar experiments, we have isolated FGF-2-CK2 complexes from the nucleus and cytoplasm of NIH3T3 cells in late G₁ (12 h) at the transition G₁/S. The serine 117 plays a pivotal role in FGF-2/CK2 interaction (13). It is remarkable to note that the FGF-2 S117A, which does not interact in the core of the cells with either the CK2 or the RSK2, has the most reduced mitogenic activity. Nevertheless, the CK2 was not associated with FGF-2-RSK2 complexes (data not shown), and FGF-2-RSK2 complexes were never detected in the cytoplasm of NIH3T3 cells and in late G₁. This suggests that the exogenously added FGF-2 is integrated in at least two distinct multiprotein complexes in the nucleus of target cells.

Recently, Hu *et al.*, have isolated RSK1-FGFR1 complexes from the nuclei of the prenatal rat brain (33). RSK1 and FGFR1 were never detected in FGF-2-RSK2 complexes *in vivo* (data not shown), and *in vitro* RSK1 does not interact with FGF-2.

The transient nuclear accumulation of FGF-2-RSK2 complexes associated with the maintenance of a high level of RSK2 activity toward the Ser-10 phosphorylation of histone H3 could represent a mechanism of direct signaling to chromatin through histone modification, which may cause a sustained chromatin remodeling (34) and/or facilitate transcription of genes related to cellular response induced by FGF-2 (35–37). Taken together, all of these findings strengthen the concept that a growing number of growth factors elicit their mitogenic response in a bifunctional manner by activating cell surface receptors and by directly associating with nuclear targets.

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REFERENCES

- Lander, E. S., Linton, L. M., Birren, B., Nusbaum, C., Zody, M. C., Baldwin, J., Devon, K., Dewar, K., Doyle, K., FitzHugh, W., Funke, R., Gage, D., Harris, K., Heaford, A., Howland, J., Kann, L., LeHocqzy, J., Levine, R., McEwan, P., McKernan, K., Meldrum, J., Mesirov, J. P., Miranda, C., Morris, W., Naylor, J., Raymond, C., Rosetti, M., Santos, R., Sheridan, A., Sougnez, C., Stange-Thomann, N., Stojanovic, N., Subramanian, A., Wyman, D., Rogers, J., Sulston, J., Ainscough, R., Beck, S., Bentley, D., Burton, J., Clee, C., Carter, N., Coulson, A., Deadman, R., Deloukas, P., Dunham, A., Dunham, I., Durbin, R., French, L., Grafham, D., Gregory, S., Hubbard, T., Humphray, S., Hunt, A., Jones, M., Lloyd, C., McMurray, A., Matthews, L., Mercer, S., Milne, S., Mullikin, J. C., Mungall, A., Plumb, R., Ross, M., Showkneen, R., Sims, S., Waterston, R. H., Wilson, R. K., Hillier, L. W., McPherson, J. D., Marra, M. A., Mardis, E. R., Fulton, L. A., Chinwalla, A. T., Pepin, K. H., Gish, W. R., Chissoe, S. L., Wendt, M. C., Delehaunty, K. D., Miner, T. L., Delehaunty, A., Kramer, J. B., Cook, L. L., Fulton, R. S., Johnson, D. L., Minx, P. J., Clifton, S. W., Hawkins, T., Branscomb, E., Predki, P., Richardson, P., Wenning, S., Slezak, T., Doggett, N., Cheng, J. F., Olsen, A., Lucas, S., Elkin, C., Uberbacher, E., Frazier, M., Gibbs, R. A., Muzny, D. M., Scherer, S. E., Bouck, J. B., Sodergren, E. J., Worley, K. C., Rives, C. M., Gorrell, J. H., Metzker, M. L., Naylor, S. L., Kucherlapati, R. S., Nelson, D. L., Weinstock, G. M., Sakaki, Y., Fujiiyama, A., Hattori, M., Yada, T., Toyoda, A., Itoh, T., Kawagoe, C., Watanabe, H., Totoki, Y., Taylor, T., Weissenbach, J., Heilig, R., Saurin, W., Artiguenave, F., Brottier, P., Bruls, T., Pelletier, E., Robert, C., Wincker, P., Smith, D. R., Doucette-Stamm, L., Rubinfeld, M., Weinstock, K., Lee, H. M., Dubois, J., Rosenthal, A., Platzer, M., Nyakatura, G., Taudien, S., Rump, A., Yang, H., Yu, J., Wang, J., Huang, G., Gu, J., Hood, L., Rowen, L., Madan, A., Qin, S., Davis, R. W., Federspiel, N. A., Abola, A. P., Proctor, M. J., Myers, R. M., Schmutz, J., Dickson, M., Grimwood, J., Cox, D. R., Olson, M. V., Kaul, R., Shimizu, N., Kawasaki, K., Minoshima, S., Evans, G. A., Athanasiou, M., Schultz, R., Roe, B. A., Chen, F., Pan, H., Ramser, J., Lehrach, H., Reinhardt, R., McCombie, W. R., de la Bastide, M., Dedhia, N., Blocker, H., Hornischer, K., Nordsiek, G., Agarwala, R., Aravind, L., Bailey, J. A., Bateman, A., Batzoglu, S., Birney, E., Bork, P., Brown, D. G., Burge, C. B., Cerutti, L., Chen, H. C., Church, D., Clamp, M., Copley, R. R., Doerks, T., Eddy, S. R., Eichler, E. E., Furey, T. S., Galagan, J., Gilbert, J. G., Harmon, C., Hayashizaki, Y., Haussler, D., Hermjakob, H., Hokamp, K., Jang, W., Johnson, L. S., Jones, T. A., Kasif, S., Kasprzyk, A., Kennedy, S., Kent, W. J., Kitts, P., Koonin, E. V., Korf, I., Kulp, D., Lancet, D., Lowe, T. M., McLysaght, A., Mikkelsen, T., Moran, J. V., Mulder, N., Pollara, V. J., Ponting, C. P., Schuler, G., Schultz, J., Slater, G., Smit, A. F., Stupka, E., Szustakowski, J., Thierry-Mieg, D., Thierry-Mieg, J., Wagner, L., Wallis, J., Wheeler, R., Williams, A., Wolf, Y. I., Wolfe, K. H., Yang, S. P., Yeh, R. F., Collins, F., Guyer, M. S., Peterson, J., Felsenfeld, A., Wetterstrand, K. A., Patrino, A., Morgan, M. J., Szustakowski, J., de Jong, P., Catanese, J. J., Osoegawa, K., Shizuya, H., Choi, S., and Chen, Y. J. (2001) *Nature* **409**, 860–921
- Prats, H., Kaghad, M., Prats, A. C., Klagsbrun, M., Lelias, J. M., Liauzun, P., Chalons, P., Tauber, J. P., Amalric, F., Smith, J. A., and Caput, D. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86**, 1836–1840
- Arnaud, E., Touriol, C., Boutonnet, C., Gensac, M. C., Vagner, S., Prats, H., and Prats, A. C. (1999) *Mol. Cell Biol.* **19**, 505–514
- Florkiewicz, R. Z., Anchin, J., and Baird, A. (1998) *J. Biol. Chem.* **273**, 544–551
- Lin, S. Y., Makino, K., Xia, W., Matin, A., Wen, Y., Kwong, K. Y., Bourguignon, L., and Hung, M. C. (2001) *Nat. Cell Biol.* **3**, 802–808
- Bouche, G., Gas, N., Prats, H., Baldwin, V., Tauber, J. P., Teissie, J., and Amalric, F. (1987) *Proc. Natl. Acad. Sci. U. S. A.* **84**, 6770–6774
- Olsnes, S., Klingenberg, O., and Wiedlocha, A. (2003) *Physiol. Rev.* **83**, 163–182
- Jans, D. A., and Hassan, G. (1998) *BioEssays* **20**, 400–411
- Peng, H., Myers, J., Fang, X., Stachowiak, E. K., Maher, P. A., Martins, G. G., Popescu, G., Berezney, R., and Stachowiak, M. K. (2002) *J. Neurochem.* **81**, 506–524
- Imamura, T., Engleka, K., Zhan, X., Tokita, Y., Forough, R., Roeder, D., Jackson, A., Maier, J. A., Hla, T., and Maciag, T. (1990) *Science* **249**, 1567–1570
- Wiedlocha, A., Farnes, P. O., Madhus, I. H., Sandvig, K., and Olsnes, S. (1994) *Cell* **76**, 1039–1051
- Bossard, C., Laurell, H., Van Den Berghe, L., Meunier, S., Zanibellato, C., and Prats, H. (2003) *Nat. Cell Biol.* **5**, 433–439
- Bailly, K., Soulet, F., Leroy, D., Amalric, F., and Bouche, G. (2000) *FASEB J.* **14**, 333–344
- Skjerpen, C. S., Nilsen, T., Wesche, J., and Olsnes, S. (2002) *EMBO J.* **21**, 4058–4069
- Soulet, F., Al Saati, T., Roga, S., Amalric, F., and Bouche, G. (2001) *Biochem. Biophys. Res. Commun.* **289**, 591–596
- Baldin, V., Roman, A. M., Bosch-Bierne, I., Amalric, F., and Bouche, G. (1990) *EMBO J.* **9**, 1511–1517
- Jensen, C. J., Buch, M. B., Krag, T. O., Hemmings, B. A., Gammeltoft, S., and Frödin, M. (1999) *J. Biol. Chem.* **274**, 27168–27176
- Bonnet, H., Filhol, O., Truchet, I., Brethenou, P., Cochet, C., Amalric, F., and Bouche, G. (1996) *J. Biol. Chem.* **271**, 24781–24787
- Bouche, G., Baldin, V., Belenguer, P., Prats, H., and Amalric, F. (1994) *Cell. Mol. Biol. Res.* **40**, 547–554
- Meisner, H., and Czech, M. P. (1991) *Curr. Opin. Cell Biol.* **3**, 474–483
- Walker, E. H., Pacold, M. E., Perisic, O., Stephens, L., Hawkins, P. T., Wymann, M. P., and Williams, R. L. (2000) *Mol. Cell* **6**, 909–919
- Alessi, D. R. (1997) *FEBS Lett.* **402**, 121–123
- Deak, M., Clifton, A. D., Lucocq, L. M., and Alessi, D. R. (1998) *EMBO J.* **17**, 4426–4441
- Thomson, S., Clayton, A. L., Hazzalin, C. A., Rose, S., Barratt, M. J., and Mahadevan, L. C. (1999) *EMBO J.* **18**, 4779–4793
- Sassone-Corsi, P., Mizzen, C. A., Cheung, P., Crosio, C., Monaco, L., Jacquot, S., Hanauer, A., and Allis, C. D. (1999) *Science* **285**, 886–891
- Poteet-Smith, C. E., Smith, J. A., Lannigan, D. A., Freed, T. A., and Sturgill, T. W. (1999) *J. Biol. Chem.* **274**, 22135–22138
- Jones, S. M., and Kazlauskas, A. (2001) *Nat. Cell Biol.* **3**, 165–172
- Faham, S., Linhardt, R. J., and Rees, D. C. (1998) *Curr. Opin. Struct. Biol.* **8**, 578–586
- Sheng, Z., Lewis, J. A., and Chirico, W. J. (2004) *J. Biol. Chem.* **279**, 40153–40160
- Plotnikov, A. N., Hubbard, S. R., Schlessinger, J., and Mohammadi, M. (2000) *Cell* **101**, 413–424
- Facciano, A., Russo, K., Facciano, A. M., De Marchis, F., Facciano, F., Ribatti, D., Aguzzi, M. S., and Capogrossi, M. C. (2003) *J. Biol. Chem.* **278**, 8751–8760
- Claus, P., Doring, F., Gringel, S., Muller-Ostermeyer, F., Fuhlrott, J., Kraft, T., and Grothe, C. (2003) *J. Biol. Chem.* **278**, 479–485
- Hu, Y., Fang, X., Dunham, S. M., Prada, C., Stachowiak, E. K., and Stachowiak, M. K. (2004) *J. Biol. Chem.* **279**, 29325–29335
- Song, M. R., and Ghosh, A. (2004) *Nat. Neurosci.* **7**, 229–235
- Cheung, P., Tanner, K. G., Cheung, W. L., Sassone-Corsi, P., Denu, J. M., and Allis, C. D. (2000) *Mol. Cell* **5**, 905–915
- Nowak, S. J., and Corces, V. G. (2000) *Genes Dev.* **14**, 3003–3013
- Zhao, J., Yuan, X., Frödin, M., and Grummt, I. (2003) *Mol. Cell* **11**, 405–413

Exogenously Added Fibroblast Growth Factor 2 (FGF-2) to NIH3T3 Cells Interacts with Nuclear Ribosomal S6 Kinase 2 (RSK2) in a Cell Cycle-dependent Manner

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