Control of CREB-binding Protein Signaling by Nuclear Fibroblast Growth Factor Receptor-1

A NOVEL MECHANISM OF GENE REGULATION*

In integrative nuclear fibroblast growth factor receptor-1 (FGFR1) signaling a newly synthesized FGFR1 translocates to the nucleus to stimulate cell differentiation and associated gene activities. The present study shows that FGFR1 accumulates and interacts with the transcriptional co-activator CREB-binding protein (CBP) in multiple domains in the developing brain and in neural progenitor-like cells in vitro, which accompanies differentiation and postmitotic growth. Cell differentiation and gene activation by nuclear FGFR1 do not require tyrosine kinase activity. Instead, FGFR1 stimulates transcription in cooperation with CBP by increasing recruitment of RNA polymerase II and histone acetylation at the active gene promoter. FGFR1 is a multifactorial protein whose N terminus interacts with CBP and C terminus with ribosomal S6 kinase 1 (RSK1). Nuclear FGFR1 augments CBP-mediated transcription by 1) releasing the CBP C-terminal domain from RSK1 inhibition and 2) activating the CBP N-terminal domain. The interaction of FGFR1 with CBP and RSK1 allows activation of gene transcription and may play a role in cell differentiation.

During ontogeny, cells in the nervous system and in other tissues multiply, grow, and differentiate under the control of a plethora of extracellular signals. Progression through developmental phases requires concerted regulation of multiple genes, which occurs at the level of sequence specific transcription factors and at chromatin locations where remodeling occurs following histone modifications (1). Central characters in coordinating these events are transcription co-activators CREB-binding protein (CBP)

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The abbreviations used are: CBP, CREB-binding protein; FGFR, fibroblast growth factor receptor; NLS, nuclear localization signal; SP, signal peptide; TK, tyrosine kinase; TH, tyrosine hydroxylase; CRE, cAMP-responsive element; CREB, CRE-binding protein; RSK, ribosomal S6 kinase; NTK, N-terminal kinase; Ab, antibody; Luc, luciferase; GST, glutathione S-transferase; BrdUrd, bromodeoxyuridine; NPC, neural progenitor cell; E23, embryonic day 23; Pd, postnatal day; IR, immunoreactivity; Bt2cAMP, dibutyryl cAMP; SNC, substantia nigra zona compacta; aa, amino acid(s); pol, polynucleotides.

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Regulation of CBP Signaling by Nuclear FGFR1

Nuclear FGFR1 plays a crucial role in regulating CBP signaling. FGFR1, when activated, can translocate to the nucleus and bind to CBP, a transcriptional co-activator. This interaction is essential for the expression of cell-type-specific genes, such as those involved in neural differentiation. The N-terminal region of FGFR1 is critical for this nuclear translocation.

FGFR1(K514A), a kinase-deficient mutant, can still translocate to the nucleus and bind to CBP, suggesting that the nuclear localization of FGFR1 is independent of its kinase activity. The nuclear FGFR1-CBP complex can activate transcriptional activity of CRE reporter construct, indicating a possible role of FGFR1 in CBP-mediated transcriptional regulation. However, the precise mechanism of how FGFR1 interacts with CBP and how this interaction modulates transcription remains to be elucidated.

The role of FGFR1 in the cell nucleus is crucial for cell differentiation and proliferation. Nuclear FGFR1 accumulation is critical for the differentiation of NPCs, medulloblastoma cells, and neuroblasts. This accumulation is necessary for the expression of neuronal markers and the induction of neuron-specific genes.

The localization of FGFR1 in the nucleus is regulated by its transmembrane domain. The atypical transmembrane domain of FGFR1 allows for dual localization in the cytoplasm and nucleus, depending on the environmental cues. This dual localization is critical for the regulation of several cellular processes, including cell proliferation, differentiation, and apoptosis.

In conclusion, nuclear FGFR1 plays a vital role in CBP-mediated transcriptional regulation, and its localization and activity are critical for the differentiation and proliferation of NPCs. Further studies are needed to elucidate the precise mechanisms and pathways of nuclear FGFR1 signaling.

Fig. 1. K514A mutation disables FGFR1 kinase activity but not the biological effects of nuclear FGFR1. A, TE671 medulloblastoma cells were transfected with FGFR1, FGFR1(K514A), or control pcDNA3.1. The cell lysates were immunoprecipitated (IP) with C-terminal FGFR1 Ab and immunoblotted (IB) with N-terminal FGFR1 McAb6 or anti-phosphotyrosine Ab. Transfected receptors were expressed at the levels similar to endogenous FGFR1 found in many neural and endocrine cells (22). The 130- and 105-kDa bands represent FGFR1 glycosylation (27).

B, individual receptor plasmids or control pcDNA3.1 (2 μg) were co-transfected with pEGFP (1 μg) to mark transfected TE671 cells. After 48 h, some TE671 cultures were incubated with 10 μM BrdUrd for an additional hour and were processed for anti-BrdUrd immunostaining (19). BrdUrd expressing and nonexpressing green fluorescent cells were counted. The combined length of processes in individual green fluorescent cells in the remaining dishes was measured 4 days after transfection. (FGFR1(K514A)-GFP)-/H9251

C, individual receptor plasmids or control pcDNA3.1 (2 μg) were co-transfected with pEGFP (1 μg) to mark transfected TE671 cells. After 48 h, some TE671 cultures were incubated with 10 μM BrdUrd for an additional hour and were processed for anti-BrdUrd immunostaining (19). BrdUrd expressing and nonexpressing green fluorescent cells were counted. The combined length of processes in individual green fluorescent cells in the remaining dishes was measured 4 days after transfection. (FGFR1(K514A)-GFP)-/H9251

D, cell lysates were incubated with 10 μM BrdUrd for an additional hour and were processed for anti-BrdUrd immunostaining (19). BrdUrd expressing and nonexpressing green fluorescent cells were counted. The combined length of processes in individual green fluorescent cells in the remaining dishes was measured 4 days after transfection. (FGFR1(K514A)-GFP)-/H9251
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factors and/or CBP. In the present study we show that the nuclear receptor associates with CBP in developing murine brain neurons and in cultured NPCs. FGFR1 activates transcription by binding to RSK1 and dissociating the inactive CBP-RSK1 complex. Nuclear FGFR1 also binds to CBP and augments CBP-mediated transcription in a process that involves recruitment of RNA pol II and histone acetylation at the core promoter of an endogenous gene. These nuclear FGFR1-driven events allow activation of gene transcription and play a role in cell differentiation.

**EXPERIMENTAL PROCEDURES**

**DNA Constructs—**pDNA3.1 plasmids expressing wild type FGFR1, FGFR1(SP−/−NLS) in which signal peptide was replaced by the SV40 large T antigen NLS, and whose tyrosine kinase-deleted mutants were described by Peng et al. (22). Kinase-inactive FGFR1(K514A) and FGFR1(K514A)(SP−/−NLS) were generated using the Stratagene QuikChange site-directed mutagenesis kit and primers as follows: gacaagttgtgGCGatgtaggagcc and egtcgctactaactCGCaacgcaacctggtgc. pCRE-luc, pAP-Luc, pNfB-luc, and pCIS-CK (lacking a cis-acting element) and pFR-Luc containing the GAL4 binding site, pFOC (112 bp region of the FGF-2 promoter, 5′-GCCATCCA-CAGTCTTCTGGG-3′; reverse, 5′-GCCATCCAACAGTCTTCTGGG-3′) or the +2245/+2765 bp region of control glyceraldehyde-3-phosphate dehydrogenase gene (forward, 5′-TCAACCATTGAGAAGGCT-3′; reverse, 5′-GCCATCCA-CAGTCTTCTGGG-3′). PCR products were resolved on 1% agarose gel and stained with ethidium bromide.

**GST Pull-down—**GST- or GST-CBP fragment fusion proteins were expressed in Escherichia coli DH5α, and the crude lysates were prepared as described by Peng et al. (30). Nuclear TE671 cell lysates were selected with GST- or GST-CBP beads and resolved by 8% SDS-PAGE for immunoblotting.

**Immunocytochemistry—**Postnatal day 4 and postnatal day 10 mice were perfused transcardially with phosphate buffered saline (pH 7.2) followed by 4% paraformaldehyde. Coronal 50-μm brain cryostat sections were incubated with rabbit polyclonal rabbit anti-C-terminal FGFR1 antibody (Santa Cruz Biotechnology, dilution 1:1000 in 10% normal goat serum) or in combination with monoclonal anti-CBP antibody (dilution 1:1000). Secondary antibodies were Cy3-conjugated goat anti-rabbit (1:600 in 10% normal goat serum) and Alexa Fluor 488 goat anti-mouse (1:150). Cultured cells were immunostained using a monoclonal N-terminal FGFR1 McAb6, with sheep polyclonal anti-phospho-Ser (364) RSK1 Ab or with rabbit polyclonal CBP Ab as described previously (16). Fluorescent staining was performed using 1) goat anti-mouse CY3, 2) goat anti-rabbit Alexa 488, or 3) donkey anti-sheep CY3 (Molecular Probes Inc., Eugene, OR). Specificity of immunostaining was ascertained with control reactions in which the primary Ab was preincubated or replaced with cognate peptides. Anti-c-myc antibody with cognate peptide as shown previously (26).2 Staining was observed using a Nikon Diaphot microscope or Bio-Rad MRC 1024 confocal microscope.

**BrdUrd Incorporation and Morphological Analysis—**Cells were transfected using Lipofectamine Plus (Invitrogen) with 0.7 μg of pGFP (to mark transfected cells) and with additional plasmids (1:3). The total amount of transfected DNA was kept constant with appropriate control vectors. Two days after transfection, some of the TE671 cultures were incubated with 10 μM BrdUrd for 1 h and were processed for anti-BrdUrd immunostaining immediately thereafter as previously described (19). Four days after transfection the total length of processes in each green fluorescent cell was measured in the remaining dishes as previously described (19). “N” refers to the number of cells scored; all experiments were repeated at least three times.

**RESULTS**

Nuclear FGFR1 Stimulates Cell Differentiation and Gene Activities in a Tyrosine Kinase Activity-independent Manner—Previous studies showed that differentiation of NPCs induced by extracellular stimuli or CAMP was mediated by endogenous FGFR1 that accumulated in the cell nucleus (19). Similar stimulation of endogenous TH or FGF-2 genes and their promoters by angiotensin II, cell depolarization, or by protein kinase C was mediated by nuclear FGFR1. These effects were mimicked by transfection of nuclear FGFR1(SP−/−NLS) and prevented by deletion of the receptor TK domain (19, 22, 23). To elucidate the mechanism of the TK domain-dependent action of nuclear FGFR1 we examined whether FGFR1 kinase activity was necessary for its biological effects. The K514A mutation prevents ATP binding and hydrolysis by FGFR1 catalytic domain and abolishes FGFR1 kinase activity (31). We confirmed the lack of FGFR1(K514A) autophosphorylation by expressing the receptor into TE671 cells, which lack detectable endogenous FGFR1. Transfected FGFR1(K514A) and the wild type receptor were

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2 Also, the specific C-terminal or McAb6 FGFR1 immunostaining was confirmed in transfection experiments using TE671 and other cells. Cells transfected with the FGFR1 but not with its membrane arrested et FGFR1(R4 mutant (16, 19, 23, 27) displayed nuclear FGFR1 immunoreactivity. Nuclear accumulation of nontruncated FGFR1 was also demonstrated after transfection of FGFR1-myc or FGFR1-Egfp using antibodies against the attached epitope and of native EGFP fluorescence in live cells (11, 12, 23).

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FIG. 2. Nuclear FGFR1 stimulates transcription in cooperation with CBP. A, FGFR1 stimulates CBP and RNA pol II binding and histone H3 acetylation at the FGF-2 core promoter. TE671 were transfected with FGFR1 (R1), FGFR1(K514A) (R1m), or pcDNA3.1 (p). Chromatin was cross-linked and immunoprecipitated with the indicated Ab. FGF-2 promoter (-211/+112 bp) and a fragment of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were amplified.

B, Fold stimulation by GAL4-fusion protein in the presence of CBP or FGFR1. C, Fold stimulation by GAL4-fusion protein in the presence of CBP or FGFR1. D, Fold stimulation of promoter activity by GAL4-fusion protein in the presence of CBP or FGFR1. E, Relative luciferase activity of various promoters in the presence of GAL4-fusion protein. F, Fold stimulation of FGF-2-Luc by pcDNA, FGFR1(SP-NLS), or FGFR1(SP-NLS)+CBP. G, % stimulation of GAPDH promoter by FGFR1(SP-NLS) in the presence of CBP and actin.
expressed at similar levels. However, only the wild type receptor was detected with anti-phosphotyrosine Ab (Fig. 1A).

Next we examined the effects of the K514A mutation on the biological activity of nuclear FGFR1(SP−/NLS). Surprisingly, differentiation of TE671 cells and other established effects of the nuclear receptor were not affected by the K514A mutation (Fig. 1, B–D). FGFR1(SP−/NLS) and kinase-inactive FGFR1(K514A)(SP−/NLS) induced the transfected cells to withdraw from the cell cycle, grow neurite-like processes (Fig. 1B), and up-regulate the endogenous catecholamine biosynthetic TH enzyme (Fig. 1C). Both (K514A)(SP−/NLS) and FGFR1(SP−/NLS) transactivated co-transfected G2F-2 and TH promoters and CRE (TH gene regulatory element) linked to the luciferase reporter (Fig. 1D).

Thus, activation of cell differentiation and transcription by nuclear FGFR1 did not require the TK activity of the receptor and, therefore, were distinct from the TK activity-dependent mitogenic effects of the cell surface FGFR1 (28). The subsequent experiments examined the mechanisms of the TK activity-independent action of nuclear FGFR1.

Nuclear FGFR1 Stimulates Transcription in Co-operation with CBP—Stimulation of cells with angiotensin II or forskolin and phorbol 12-myristate 13-acetate increased the interaction of endogenous FGFR1 with the G2F-2 gene promoter in in vitro DNA-protein binding assays (22). To determine if in vivo nuclear FGFR1 may itself associate with the promoter and/or affect binding of proteins involved in transcription, we performed cross-linked chromatin immunoprecipitations. As a model we used the G2F-2 gene whose activation by transfected FGFR1 (similar to the activation by angiotensin II, cAMP, or protein kinase C) is mediated by intracellular, but not the cell membrane-associated receptor (22). Transfection of FGFR1 induced FGFR1 and CBP association with the G2F-2 core promoter (Fig. 2A) (the co-association of endogenous FGFR1 and CBP with G2F-2 promoter was also found (not shown)). Furthermore, in cells transfected with the kinase-active or -inactive receptor, the amount of RNA pol II and acetylated H3 associated with the G2F-2 promoter increased compared with the cells transfected with control vector. These changes were not observed in the control glyceraldehyde-3-phosphate dehydrogenase gene locus.

To examine whether promoter-associated FGFR1 may activate transcription and therefore act as transcription factor or co-activator, we transfected TE671 cells with chimeric FGFR1 or FGFR1(NT−) (lacking the N-terminal region upstream from the TK domain) fused to the GAL4 DNA-binding domain (1–147 aa). Neither GAL4/FGFR1 nor GAL4/FGFR1(NT−) stimulated luciferase transcription from the GAL4-binding element. A partial transcription inhibition was observed compared with nonfused GAL4 (Fig. 2B, left).

Nuclear FGFR1 mediated activation of the TH promoter in stimulated cells, which involved the CREB/CBP-binding CRE element (23). Hence, we examined whether GAL4/FGFR1 may activate transcription in cooperation with CBP. Although CBP alone had no effect on luciferase activity, it stimulated transcription in a synergistic manner with GAL4/FGFR1, but not with GAL4/FGFR1(NT−) (Fig. 2B, right) even though both fusion proteins were expressed at the similar levels (not shown). Thus, promoter-associated FGFR1 can stimulate transcription in cooperation with CBP. The N-terminal domain of FGFR1 was essential for this co-activation.

CBP-dependent activation of transcription by nuclear FGFR1 was verified in an experiment in which the receptor was co-transfected with CBP fused to GAL4. GAL4/CBP-mediated transcription was increased severalfold by FGFR1(SP−/NLS), whereas GAL4/CREB- and GAL4/c-Jun-mediated transcription was only marginally affected (Fig. 2C). Nuclear FGFR1 augmented CBP-mediated transcription in a kinase activity-independent manner (Fig. 2D). FGFR1(TK−)(SP−/NLS), lacking the TK, failed to stimulate GAL4/CBP-mediated transcription. Thus both the TK and the N-terminal FGFR1 domains were essential for co-stimulation of transcription with CBP.

The ability of nuclear FGFR1 to activate GAL4/CBP-mediated transcription prompted us to examine whether nuclear FGFR1 may transactivate the typical CBP-regulated promoter elements (2). Indeed, FGFR1(SP−/NLS) transactivated CRE as well as AP1 (TPA-responsive element) and NFκB-binding elements (Fig. 2E) similar to the natural TH and G2F-2 promoters (Fig. 1D). The TH and G2F-2 promoters were synergistically transactivated by co-transfected FGFR1(SP−/NLS) and CBP (Fig. 2F).

The involvement of endogenous CBP in the stimulation of transcription by nuclear FGFR1 was confirmed by depleting CBP with antisense CBP RNA (Fig. 2G). pBl-G plasmid expressing antisense CBP reduced the transactivation of CRE-Luc or TH-Luc by FGFR1(SP−/NLS). Thus, nuclear FGFR1 and CBP stimulated transcription in an interactive manner, and endogenous CBP was involved in transcriptional activation by nuclear FGFR1.

Interaction of Nuclear FGFR1 with CBP—To determine if FGFR1 may interact with CBP in a TK activity-independent manner we transfected TE671 cells with FGFR1 or FGFR1(K514A). FGFR1 and FGFR1(K514A) were detected in the immunoprecipitates generated with the FGFR1 C-terminal Ab or with the CBP antibody (Fig. 3A). The CBP Ab also co-immunoprecipitated FGFR1(TK−) (Fig. 3B). Thus, the FGFR1 N-terminal region associates with CBP. CBP associated predominantly with the hypoglycosylated form of FGFR1 (105 kDa, Fig. 3A) and FGFR1(TK−) (65 kDa, Fig. 3B). The CBP-FGFR1 interaction was verified by immunoprecipitation with the FGFR1 C-terminal Ab followed by immunoblotting with the CBP Ab. No co-immunoprecipitation was observed in cells transfected with control pcDNA3.1 showing that the FGFR1 Ab did not directly precipitate CBP (Fig. 3A).

The interaction between endogenous nuclear FGFR1 and CBP was confirmed by analyzing subcellular fractions from the E23
rodent brain. At this time point, FGFR1 was shown to be present both in the cytoplasm and nuclei (16). Only nuclear receptor was co-immunoprecipitated with the CBP antibody (Fig. 3C).

Both CBP (32) and FGFR1 (19, 33) concentrate predominantly within the speckle-like nuclear domains. To determine if endogenous FGFR1 and CBP co-localize and thus may interact within the same specific domains, we analyzed FGFR1 and CBP immunoreactivity (IR) in NPCs and in developing brain.

In cultured NPCs, similar as in other cells (11), increases in endogenous FGFR1 content accompanied by nuclear accumulation of FGFR1 can be induced by Bt2cAMP and other stimuli that inhibit proliferation and induce outgrowth of cellular processes (16, 19). Proliferating NPCs expressed CBP similar to the Bt2cAMP-differentiated cells (Fig. 3D). These cells showed limited nuclear FGFR1-IR and only few CBP-IR speckles co-stained for FGFR1. A marked nuclear accumulation of FGFR1

FIG. 3. Co-immunoprecipitation and co-localization of nuclear FGFR1 and CBP. A, FGFR1 and FGFR1(K514/A) bind to CBP. TE671 were transfected with indicated plasmids. Cell extracts were analyzed directly by immunoblotting or were immunoprecipitated (Ip) with FGFR1 or CBP Ab and immunoblotted (Ib) with FGFR1 McAb6 or CBP Ab. Arrows point to glycosylation forms of full-length or tyrosine kinase-deleted receptors and to CBP. B, FGFR1(TK−) binds to CBP. TE671 cells were transfected with FGFR1 or FGFR1(TK−). Cell extracts were co-immunoprecipitated with CBP Ab and immunoblotted with FGFR1 McAb6 or were directly subjected to Western analysis with FGFR1 McAb6. C, association of endogenous FGFR1 and CBP in the nuclei of the prenatal rat brain. Nuclear (N) and cytoplasmic (C) fractions (1 mg) of the whole brain at embryonic day 21 were immunoprecipitated with the CBP polyclonal Ab, FGFR1 C-terminal Ab or control polyclonal Ab. All proteins were immunoblotted with the N-terminal FGFR1 McAb6. D) Cultures of neural progenitor-like cells (NPCs) were treated with 0.1 mM Bt2cAMP for 48 h and co-immunostained with FGFR1 McAb6 (+ anti-mouse-Alexa 488) and with rabbit CBP (+ goat-anti rabbit-CY3). Confocal sections through the middle of the nuclei are shown. Merged images - FGFR1 and CBP immunoreactive pixels co-localize within CBP-rich nuclear foci. E, nuclear FGFR1 accumulation correlates with postmitotic neuronal development in vivo. Brain sections were incubated with rabbit C-terminal FGFR1 Ab/goat anti-rabbit-Cy3 Ab. At postnatal day (Pd) 4 FGFR1-IR was predominantly cytoplasmic in subventricular zone (SVZ) (i), nuclear and cytoplasmic in diencephalon (ii), and predominantly nuclear substantia nigra zona compacta (SNc) (iii). At Pd10 FGFR1-IR in SNc was predominantly cytoplasmic (iv). Panel v shows double immunostaining of SNc at Pd4 with C-terminal FGFR1 Ab/goat ant-rabbit-Cy3 and with monoclonal CBP Ab/goat anti-mouse-Alexa Fluor 488. Images represent confocal sections through approximately the middle of the nucleus. The right panel shows 10-fold enlarged nucleus (*).
induced by Bt2cAMP was accompanied by co-localization of FGFR1-IR and CBP-IR pixels within the CBP-rich 0.5–2 μm³ nuclear speckles. A similar co-localization was also observed in Bt2cAMP-treated human fetal brain-derived NPCs and rat hippocampal NPCs (not shown). To ascertain whether nuclear accumulation of FGFR1 and its co-localization with CBP follow a similar pattern in the developing brain, we analyzed FGFR1-IR at postnatal day (Pd) 4, a point in development at which cells in different brain regions are present in different developmental stages. Endogenous FGFR-IR analysis was performed in the subventricular zone, which contains rapidly proliferating transit-amplifying progenitor cells. This analysis was also performed at different distance from the subventricular zone in regions containing differentiating and maturing neurons (Fig. 3E). In the subventricular zone FGFR1-IR was concentrated predominantly outside the nuclei (Fig. 3E, panel i), whereas in the diencephalon FGFR1-IR was found both in the cytoplasm and nuclei (panel ii). In the more posterior SNc region, where neurons develop telencephalic projections and synaptic contacts, FGFR1-IR was predominantly nuclear (panel iii). Development of the SNc projection is complete within the second postnatal week (34). At that time (Pd10; Fig. 3E, panel iv) and in the adult brain (not shown) FGFR1-IR was again predominantly cytoplasmic. Double immunostaining revealed that in the SNc at Pd4, the FGFR1 and CBP pixels were co-localized within the nuclear speckles (panel v). These results conformed to the observation in vitro that showed nuclear accumulation of FGFR1 during G0/G1 and its depletion in cells with established intercellular contacts (27).

**FIGFR1 Prevents the CBP-RSK1 Interaction and Activates CBP through RSK1-dependent and -independent Mechanisms—** Despite FGFR1(TK−) binding to CBP, the truncated receptor failed to stimulate GAL4/CBP. We hypothesized that nuclear FGFR1 may act as a scaffold whereby its N terminus binds CBP and the C-terminal TK interacts with the one or more proteins that control CBP functions. One candidate protein was RSK1, which binds to CBP in mitogen-stimulated cells (10). RSK1 can also bind to endogenous nuclear FGFR1 in vivo in developing brain at the time when the CBP-FGFR1 binding was observed (Fig. 3B) and in vitro in differentiating NPCs (17). Fig. 4A confirms co-immunoprecipitation of RSK1 with CBP in serum-stimulated cells. Because TE671 lacked detectable endogenous FGFR1, the RSK1-CBP interaction was likely FGFR1-independent.

Next we examined the effects of RSK1 on CBP-dependent transcription. Transfected RSK1 inhibited transcription from the TH and FGF-2 promoters as well as from the CRE, TPA-responsive element, or NFκB-binding elements (Fig. 4B). The mechanisms of this inhibition were examined using sequence-specific transcription factors or CBP fused to GAL4 (1–147). Transcription mediated by GAL4/CREB and GAL4/-c-Jun was stimulated by RSK1, but not by its kinase-inactive (RSK1−) K112R/K464R mutant (Fig. 4C). In contrast, GAL4/CBP-mediated transcription was stimulated by kinase-inactive RSK1(−) and inhibited by active RSK1. Thus, while RSK1 activated CREB and c-Jun, it inhibited the transcription co-activator CBP.

To determine the dynamics of the FGFR1, CBP, and RSK1 interactions and their functional significance, we first analyzed their co-localization in proliferating and differentiating NPCs. In proliferating cells, CBP-IR pixels co-localized with RSK1-IR pixels within the CBP-rich nuclear speckles (Fig. 5A). Bt2cAMP-induced nuclear FGFR1 accumulation within the CBP speckles was accompanied by an emergence of co-localized FGFR1-IR and CBP-IR pixels (see also Fig. 3D), co-localization of FGFR1-IR and RSK1-IR pixels and by a loss of CBP-IR and CBP speckles was accompanied by an emergence of co-localized FGFR1-IR and RSK1-IR pixels and by a loss of CBP-IR and CBP speckles.

**FIG. 4. RSK1 binds to CBP and inhibits CBP-mediated transcription.** A, TE671 were cultured without or with 15% fetal bovine serum for 48 h. Extracts were directly subjected to Western immunoblotting with CBP Ab or RSK1 Ab or were immunoprecipitated with CBP Ab and immunoblotted with RSK1 Ab. B, overexpressed RSK1 inhibited TH and FGF-2 promoters and CRE, NFκB, and AP1 enhancers. TE671 cells were transfected with 0.5 μg of different reporter genes and with 1 μg of pKH3RSK1 or its control vector pRK7. Luciferase activity was expressed relative to pRK7 (100%). C, the opposite effects of RSK1 on CREB, AP1, and CBP. TE671 cells were transfected with gal4-luc, plasmids expressing GAL4, or individual GAL4 fusion proteins and with pKH3RSK1, pKH3RSK1(K112/464R) (expresses inactive RSK1), or control pRK7 vector.

RSK1-IR co-localization (Fig. 5A). Hence, in nuclei that have accumulated FGFR1, CBP and RSK1 may no longer interact and formation of separate FGFR1-RSK1 and FGFR1-CBP complexes is promoted.

To ascertain whether FGFR1 accumulation dissociates the nuclear CBP-RSK1 complex we transfected TE671 cells with FGFR1 or FGFR1(K514/A). Binding of transfected FGFR1 and FGFR1(K514/A) to endogenous CBP was shown in Fig. 3A and to RSK1 was shown previously (16). The interaction between endogenous RSK1 and CBP was followed by co-immunoprecipitation. As shown on Fig. 5B the binding between CBP and RSK1 (lane 1) was prevented by transfection of FGFR1 or FGFR1(K514/A) (lanes 2 and 3) indicating that accumulating nuclear FGFR1 can eliminate the RSK1-CBP complex. How-
ever, when additional RSK1 was transfected, the formation of the RSK1-CBP complex was reinstated in the presence of co-transfected FGFR1 (Fig. 5B, lane 4). To confirm that FGFR1 and RSK1 interact with CBP in a mutually exclusive manner we performed CBP-affinity selection assays for FGFR1 and RSK1 on a series of glutathione S-transferase (GST)-CBP fusion polypeptides. Fragments of CBP fused to GST were incubated with nuclear extracts of TE671 cells transfected with FGFR1, FGFR1(K514/A), or control plasmids as indicated. Cell lysates were first immunoprecipitated with CBP Ab or were directly immunoblotted with RSK1 Ab. C, GST/CBP fusion proteins were expressed in E. coli and isolated with glutathione-Sepharose 4B beads. Cell lysates from TE671 transfected with FGFR1 or FGFR1(TK−) or with control vector were incubated with the beads. The eluted proteins were resolved on SDS-PAGE and immunoblotted with FGFR1 McAb6 Ab or anti-RSK1 Ab. D, FGFR1 TK and RSK1 NTK bind in mammalian two-hybrid assay. GAL4 (1–147 aa) was fused to FGFR1(NT−) (isolated FGFR1 TK domain), and VP16 was fused to full-length (RSK1) or NTK-truncated RSK1(NT−). TE671 cells were transfected with a gal4-luciferase and with GAL4 fusion protein and VP16 fusion protein or with nonfused control plasmids. The fold luciferase stimulation was calculated relative to cells transfected with the control vectors.

FIG. 5. FGFR1 prevents the CBP-RSK1 interaction and activates CBP through RSK1-dependent and-independent mechanisms. A, proliferating and Bt-cAMP-treated (24 h) differentiating NPCs were co-immunolabeled with FGFR1 McAb6 (plus anti-mouse-CY3); rabbit anti-CBP (plus goat-anti rabbit-Alex 488), and sheep anti-phospho-RSK1 (plus donkey anti-sheep-CY5). Examples of confocal sections through the middle of the nuclei are shown. Co-localized pixels are shown yellow (RSK1 and red and CBP green) or purple (FGFR1 are red and RSK1, blue). B, FGFR1 disrupts formation of the endogenous RSK1-CBP complex. TE671 were transfected with FGFR1 or FGFR1(K514/A) (8 μg) and FLAG/RSK1 (2 μg) or control plasmids as indicated. Cell lysates were first immunoprecipitated with CBP Ab or were directly immunoblotted with RSK1 Ab. C, GST/CBP fusion proteins were expressed in E. coli and isolated with glutathione-Sepharose 4B beads. Cell lysates from TE671 transfected with FGFR1 or FGFR1(TK−) or with control vector were incubated with the beads. The eluted proteins were resolved on SDS-PAGE and immunoblotted with FGFR1 McAb6 Ab or anti-RSK1 Ab. D, FGFR1 TK and RSK1 NTK bind in mammalian two-hybrid assay. GAL4 (1–147 aa) was fused to FGFR1(NT−) (isolated FGFR1 TK domain), and VP16 was fused to full-length (RSK1) or NTK-truncated RSK1(NT−). TE671 cells were transfected with a gal4-luciferase and with GAL4 fusion protein and VP16 fusion protein or with nonfused control plasmids. The fold luciferase stimulation was calculated relative to cells transfected with the control vectors.
stimulation by FGFR1(SP−/H11002−/NLS) (not shown). The N- and C-terminal CBP fragments can act as autonomous domains that mediate transactivation by specific signals and have been used to analyze diverse signaling mechanisms (36). Hence, to further determine how nuclear FGFR1 activates CBP-mediated transcription and the role of RSK1, we tested whether FGFR1 activates the CBP C-terminal module (1678–2441 aa) that binds RSK1 but not FGFR1 or the CBP N-terminal module (460–650 aa) that binds FGFR1 but not RSK1. We chose the 460- to 650-aa region because activity of further upstream CBP regions were reported to be affected by RSK1 (37). Both GAL4/CBP-(460–650) and GAL4/CBP-(1678–2441) increased gal4-luciferase transcription, and their effects were augmented by FGFR1(SP−/H11002−/NLS) (Fig. 6B). FGFR1(SP−/H11002−/NLS) stimulation of GAL4/CBP(1678–2441), but not of GAL4/CBP(460–650), was blocked by transfected RSK1 (Fig. 6B).

CBP, RSK1, and Nuclear FGFR1 Interactions in Cell Differentiation—The significance of CBP-RSK association/dissociation was established by Nakajima et al. (10) who showed that transfection of the RSK1 fragment that affects CBP-RSK1 interaction influences cell differentiation. We used a similar approach (transfection of RSK1) to examine whether nuclear FGFR1-induced differentiation may be depended on changes in CBP-RSK1 interaction. As a model we used NPCs that, when treated with Bt2cAMP or transfected with FGFR1(SP−/NLS), exit the cell cycle and grow long cellular processes, a response mediated by nuclear FGFR1 (16, 19, 24).

First we verified that cAMP- or CBP-induced differentiation is affected by RSK1. Examples of proliferating NPCs with no process or with short processes and the outgrowth of processes induced by different treatments are shown in Fig. 7. Bt2cAMP treatment or transfection of CBP increased the total length of cell processes 2- to 3-fold. These effects were prevented by transfection of RSK1, which itself did not affect cell morphology. Hence, the effects of RSK1 on cell differentiation correlated with RSK1 inhibition of CBP and not with its activation of CREB. In parallel transfections, FGFR1(SP−/NLS) or FGFR1(K541/A)(SP−/NLS) were tested, each inducing NPCs differentiation (Fig. 7) similar to cAMP or CBP. Activation of RSK1 by FGFR1 requires TK activity (16). In contrast, kinase-inactive FGFR1 receptor can still bind to RSK1 and dissociate the CBP-RSK1 complex (Figs. 3A and 5B). Fig. 7 shows that FGFR1(SP−/NLS)- as well as FGFR1(K541/A)(SP−/NLS)-induced NPC differentiation were prevented by RSK1. Thus, the inhibition by overexpressed RSK1 may reflect specifically the TK-activity-independent interaction with FGFR1.

DISCUSSION

Earlier studies in this laboratory demonstrated that the nuclear accumulation of endogenous FGFR1 was essential for cAMP- or BMP-7-induced NPC differentiation in proliferating NPCs and related cells (11). Endogenous nuclear FGFR1 was also shown to act as a broad-function gene transducer through which many surface receptors and signaling pathways stimulate transcription. These biological responses can be modeled by transfection of FGFR1(SP−/NLS), thereby demonstrating that the nuclear accumulation of the receptor is sufficient to stimulate gene activities and cell development. The present findings that transfection of FGFR1(SP−/NLS) caused TE671 medulloblastoma and NPCs to withdraw from the cell cycle, grow neurite-like processes, and express neuron-specific genes further establish the role of nuclear FGFR1 as a molecular
switch that turns off cell proliferation and turns on differentiation and postmitotic growth. Surprisingly, induction of cell differentiation and gene activation by nuclear FGFR1 did not require TK activity (even though it required the TK domain (19, 22, 23)), suggesting a distinct mechanism from those involved in the mitogenic action of membrane-bound FGFR1 (28).

Nuclear FGFR1-mediated activation of the TH promoter by angiotensin II and other stimuli involved the CREB/CBP-binding CRE (23). In the present study, the involvement of endogenous CBP in nuclear FGFR1 functions was established using antisense CBP RNA, which depleted CBP and diminished gene transactivation by the nuclear receptor. Chromatin immunoprecipitation assays showed that FGFR1 itself associated in vivo with the targeted promoter and increased CBP binding (Fig. 2A) conforming to the earlier findings that endogenous nuclear FGFR1 can associate with the promoter in vitro (22). However, chimeric GAL4/FGFR1 lacked an autonomous transcription activating function, but acquired such a function through cooperation with the transcriptional co-activator CBP. This synergistic activation can be attributed to nuclear FGFR1 activation of CBP-mediated transcription as demonstrated by its stimulation of GAL4/CBP and by the synergistic co-activation of transcription by CBP and FGFR1(SP−/NLS). The FGFR1-CBP cooperation was further shown by their co-association observed in GST pull-down and co-immunoprecipitation assays. This interaction appears to occur specifically within the nuclear CBP-rich speckles (32), which like the FGFR-rich speckles (33) are sites of active gene transcription, but not replication.

The CBP binding domain in FGFR1 is located upstream from the TK. Still, the TK domain was required for receptor stimulation of CBP-mediated transcription. Initially, we hypothesized that the nuclear receptor acts as a scaffold whereby its N terminal binds CBP, whereas the C-terminal TK brings one or more proteins that control CBP functions. One candidate protein was pp90 RSK1, previously found to interact with TK domain of FGFR1 (16). RSKs are serine/threonine kinases in the mitogenic signaling pathway. RSKs phosphorylate a large number of sequence-specific transcription factors, including CREB, c-Fos, c-Jun, Nur77, and serum response factor (38) and, therefore, are presumed to stimulate transcription. Our findings that overexpressed RSK1 activated GAL4/CREB and GAL4/c-Jun are consistent with this model. However, transfected RSK1 inhibited GAL4/CBP activity. RSK1 also inhibited CREB-, c-Jun-, or NfκB-mediated promoter activation, which requires endogenous CBP. Therefore, the phosphorylation of CREB and other sequence-specific transcription factors by RSK1, even though it carries the potential to activate transcription, such potential may not be realized due to CBP inhibition.

Nakajima et al. (10) showed that interaction between endogenous CBP and RSK1 may lead to the CBP inhibition. Our finding that kinase inactive RSK1(−) or NfκB−, which can bind to CBP (10), increased CBP activity confirms that CBP is under inhibition by endogenous RSK1. CBP contains an RSK consensus phosphorylation site (RRLS) at Ser-1772 (39). The present study shows that RSK1 inhibition of CBP depends on RSK1 catalytic activity. Thus, CBP inhibition could reflect its phosphorylation by RSK1.

Activation of cAMP-responsive genes by forskolin required dissociation of the RSK1-CBP complex (10). Consistent with this model we found that nuclear accumulation of endogenous FGFR1 in NPCs and transfected FGFR1 in TE671 prevents the RSK1-CBP interaction while promoting new FGFR1-RSK1 and
FGFR1-CBP complexes. This effect is due to the competition between the FGFR1 TK domain and the CBP C-terminal region for binding to the RSK1 NTK. These results do not support the scaffold model in which nuclear FGFR1 bound simultaneously and brought together CBP and RSK1. Instead, our results favor a "competitor" model in which CBP and FGFR1 bind to and compete for the RSK1 NTK domain. Because separate N-terminal and C-terminal FGFR1 domains are involved in the interaction with CBP and RSK1, respectively, a steric hindrance could prevent the formation of a three-way CBP-FGFR1-RSK1 complex. CBP, RSK1, and FGFR1 remain in an equilibrium in which an increase in [FGFR1] disassociated CBP-RSK1, whereas an increase in [RSK1] promoted formation of the RSK1-CBP complex (Fig. 8A). As shown in transcription assays, these changes in the RSK1-CBP interaction caused activation and inhibition of CBP-mediated transcription, respectively. Additionally, the interactions among FGFR1, CBP, and RSK1 affect cell differentiation and may serve as a pivotal step in cAMP-induced nuclear FGFR1-mediated NPC differentiation. The interaction of FGFR1 with CBP in developing brain (Fig. 3B) was observed at the same time as the interaction of nuclear FGFR1 with RSK1 (16) indicating that these mechanisms operate during brain development. Nuclear accumulation of FGFR1 and its interaction with CBP in vivo and in vitro occur specifically in cells that exit the cell cycle. Recently, we found that ectopically expressed nuclear FGFR1 stimulated proliferating subventricular zone cells in mouse brain to exit the cell cycle (similar to cultured NPCs). Thus, interactions of nuclear FGFR1 with CBP and RSK1 may serve as a developmental switch that controls a cell transition from the proliferating to the differentiating stage. This switch offers a potential target for an anti-cancer therapy (see Fig. 1B).

Even though the inhibition of GAL4/CBP by RSK1 indicated that the suppression of CBP function is a general RSK1 property, activation of some genes may require formation of the CBP-RSK complex. In the Ras-responsive c-fos gene, RSK-CBP binding promotes transcription activation (10). CBP may also form complexes with RSK1 and simultaneously with some sequence-specific transcription factors like ER81, where one CBP function is to facilitate the interaction and activation of ER81 by RSK1 (37). The dynamic assembly/dissociation of the CBP-RSK1 complex and its regulation by nuclear FGFR1 could serve to channel the thrust of cell stimulation to different gene sets and induce different developmental stages.

Releasing CBP from RSK1 inhibition is not the only mechanism through which FGFR1 can activate CBP-mediated transcription. CBP and p300 are modular proteins, and their different fragments have autonomous co-activator functions (36, 37). Nuclear FGFR1 augmented transcription mediated by GAL4/CBP-(451–689), which lacks the RSK1 binding domain, however, unlike the FGFR1 activation of full-length CBP or of its RSK1-binding C-terminal fragment, the activation of CBP-(451–689) is not affected by overexpressed RSK1. Thus, the second mechanism could involve RSK1-independent CBP activation by nuclear FGFR1. Together, our findings indicate that in serum- or other mitogen-stimulated proliferating cells, in which CBP is arrested in a complex with RSK1, nuclear FGFR1 activates CBP in a two-step manner: 1) by binding of the nuclear FGFR1 TK domain to the N-terminal kinase of RSK1 and disrupting the inactive CBP-RSK1 complex and 2) by binding of the FGFR1 N-terminal domain to the N-terminal region of CBP and activating CBP-mediated transcription (Fig. 8A). CBP-dependent transcription activation by nuclear FGFR1 leads to the recruitment of RNA pol II and histone acetylation, indicating that the receptor stimulates both CBP functions.

The role of CBP in transcriptional regulation could be described as that of a gating factor (40). When CBP is inactive, activation of specific transcription factors by extracellular stimuli may not suffice to effectively increase gene transcription, thereby preventing cells from responding to external stimuli. To explain the function of nuclear FGFR1 we propose a “feed-forward-and-gate” signaling module in which stimulation of diverse membrane receptors initiates classic signaling cascades that transmit signals to sequence-specific transcription factors (Fig. 8B). Nuclear accumulation of FGFR1, induced by the same surface receptors, feeds the signal forward to CBP, facilitates gene activation, and enables activation of the CBP-regulated developmental gene programs. The interaction of nuclear FGFR1 with CBP and with its partner protein, RSK1, may serve as a switch that controls the transition between CBP-regulated cell developmental stages and brain development. It remains to be determined if
the developmental functions of nuclear FGFR2 (20, 21) may involve similar mechanisms.

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REFERENCES
1. Emerson, B. M. (2002) Cell 109, 267–270
2. Goodman, R. H., and Smolik, S. (2000) Genes Dev. 14, 1553–1577
3. Impey, S., Fong, A. L., Wang, Y., Cardinaux, J. R., Fass, D. M., Obrietan, K., Wayman, G. A., Storm, D. R., Soderling, T. R., and Goodman, R. H. (2002) Neuron 34, 235–244
4. Zanger, K., Cohen, L. E., Hashimoto, K., Radovick, S., and Wondisford, F. E. (1999) Mol. Endocrinol. 13, 267–278
5. Chevillard-Briet, M., Trouche, D., and Vandel, L. (2002) EMBO J. 21, 5457–5466
6. Xu, W., Chen, H., Du, K., Asahara, H., Tini, M., Emerson, B. M., Montminy, M., and Evans, R. M. (2001) Science 294, 2507–2511
7. Horvai, A. E., Xu, L., Korzus, E., Brard, G., Kalafus, D., Mullen, T. M., Rose, D. W., Rosenfeld, M. G., and Glass, C. K. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1074–1079
8. Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S. C., Heyman, R. A., Rose, D. W., Glass, C. K., and Rosenfeld, M. G. (1996) Cell 85, 405–414
9. Waltner-Law, M., Daniels, M. C., Sutherland, C., and Granner, D. K. (2000) J. Biol. Chem. 275, 31847–31856
10. Nakajima, T., Fukamizu, A., Takahashi, J., Gage, F. H., Fisher, T., Blenis, J., and Miyawaki, A. (1996) Cell 86, 465–474
11. Stachowiak, M. K., Fang, X., Myers, J. M., Dunham, S. M., Berezney, R., Maher, P. A., and Stachowiak, E. K. (2003) J. Cell. Biochem. 90, 682–691
12. Myers, J. M., Martino, G. G., Ostrowski, J., and Stachowiak, M. K. (2003) J. Cell Biochem. 88, 1273–1291
13. Reilly, J. F., and Maher, P. A. (2001) J. Cell Biol. 152, 1307–1312
14. Reilly, J. F., Mizukoshi, E., and Maher, P. A. (2004) DNA Cell Biol. 23, 538–548
15. Dunham, S. M., Pudavar, H. E., Prasad, P. N., and Stachowiak, M. K. (2004) J. Phys. Chem. B 108, 10540–10546
16. Hu, Y., Fang, X., Dunham, S. M., Prada, C., Stachowiak, E. K., and Stachowiak, M. K. (2004) J. Biol. Chem. 279, 29325–29335
17. Clarke, W. E., Berry, M., Smith, C., Kent, A., and Logan, A. (2001) Mol Cell Neurosci. 17, 17–30
18. Gonzalez, A. M., Berry, M., Maher, P. A., Logan, A., and Baird, A. (1995) Brain Res. 701, 201–226
19. Stachowiak, E. K., Fang, X., Myers, J., Dunham, S., and Stachowiak, M. K. (2003) J. Neurochem. 84, 1296–1312
20. Sabbieiti, M. G., Marchetti, L., Gabrielli, M. G., Menghi, M., Materazzi, S., Menghi, G., Raissi, L. G., and Hurley, M. M. (2002) Cell Tissue Res. 319, 267–278
21. Schmahl, J., Kim, Y., Colvin, J. S., Ornitz, D. M., and Capel, B. (2004) Development 131, 3627–3636
22. Peng, H., Moffett, J., Myers, J., Fang, X., Stachowiak, E. K., Maher, P., Kratz, E., Hines, J., Fluharty, S. J., Mizukoshi, E., Bloom, D. C., and Stachowiak, M. K. (2001) Mol. Biol. Cell 12, 449–462
23. 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
24. Buzanska, L., Machaj, E. K., Zahlocka, B., Pojda, Z., and Domanska-Janik, K. (2002) J. Cell Sci. 115, 2131–2138
25. Bryant, D. M., Wylie, F. G., and Stow, J. L. (2005) Mol. Biol. Cell 16, 14–23
26. Stachowiak, M. K., Maher, P. A., Joy, A., Mordechai, E., and Stachowiak, E. K. (1996) Mol. Biol. Cell 7, 1299–1317
27. Stachowiak, M. K., Moffett, J., Maher, P., Tucholski, J., and Stachowiak, E. K. (1997) Mol. Neurobiol. 15, 257–283
28. Mohammadi, M., Dikic, I., Sorokin, A., Burgess, W. H., Jaye, M., and Schlessinger, J. (1996) Mol. Cell. Biol. 16, 977–989
29. Hanneken, A., Maher, P. A., and Baird, A. (1995) J. Cell Biol. 126, 1221–1228
30. Peng, H., He, H., Hay, J., and Ruyechan, W. T. (2003) J. Biol. Chem. 278, 30668–30675
31. Bellot, F., Crumley, G., Kaplow, J. M., Schlessinger, J., Jaye, M., and Dionne, C. A. (1991) EMBO J. 10, 2849–2854
32. von Mikecz, A., Zhang, S., Montminy, M., Tan, E. M., and Hemmerich, P. (2000) J. Cell Biol. 150, 265–273
33. Somanathan, S., Stachowiak, E. K., Siegel, A. J., Stachowiak, M. K., and Berezney, R. (2003) J. Cell. Biochem. 90, 856–869
34. Baker, H., Jhe, T. H., and Reis, D. J. (1982) Brain Res. 256, 157–165
35. Merienne, K., Pannetier, S., Harel-Bellan, A., and Sassone-Corsi, P. (2001) Mol. Biol. Cell 21, 7089–7096
36. Swope, D. L., Mueller, C. L., and Chrivia, J. C. (1996) J. Biol. Chem. 271, 20138–20145
37. Wu, J., and Janknecht, R. (2002) J. Biol. Chem. 277, 42669–42679
38. Rons, P. F., and Blenis, J. (2004) Microbiol. Mol. Biol. Rev. 68, 320–344
39. Liu, Y. Z., Chrivia, J. C., and Latchman, D. S. (1998) J. Biol. Chem. 273, 32400–32407
40. Shaywitz, A. J., and Greenberg, M. E. (1999) Annu. Rev. Biochem. 68, 821–861
Control of CREB-binding Protein Signaling by Nuclear Fibroblast Growth Factor Receptor-1: A NOVEL MECHANISM OF GENE REGULATION
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