Journal of Cellular Biochemistry 90:662–691 (2003)

Integrative Nuclear FGFR1 Signaling (INFS) as a Part of a Universal ''Feed-Forward-And-Gate'' Signaling Module That Controls Cell Growth and Differentiation

Michal K. Stachowiak,[1]* Xiaohong Fang,[1] Jason M. Myers,[1] Star M. Dunham,[1] Ronald Berezney,[2] Pamela A. Maher,[3] and Ewa K. Stachowiak[1]*

[1]Molecular and Structural Neurobiology and Gene Therapy Program, Department of Pathology and Anatomical Sciences, New York
[2]Department of Biological Sciences, State University of New York at Buffalo, Buffalo, New York
[3]The Scripps Research Institute, La Jolla, California

Abstract A novel signaling mechanism is described through which extracellular signals and intracellular signaling pathways regulate proliferation, growth, differentiation, and other functions of cells in the nervous system. Upon cell stimulation, fibroblast growth factor receptor-1 (FGFR1), a typically plasma membrane-associated protein, is released from ER membranes into the cytosol and translocates to the cell nucleus by an importin-β-mediated transport pathway along with its ligand, FGF-2. The nuclear accumulation of FGFR1 is activated by changes in cell contacts and by stimulation of cells with growth factors, neurotransmitters and hormones as well as by a variety of different second messengers and thus was named integrative nuclear FGFR1 signaling (INFS). In the nucleus, FGFR1 localizes specifically within nuclear matrix-attached speckle-domains, which are known to be sites for RNA Pol II-mediated transcription and co-transcriptional pre-mRNA processing. In these domains, nuclear FGFR1 colocalizes with RNA transcription sites, splicing factors, modified histones, phosphorylated RNA Pol II, and signaling kinases. Within the nucleus, FGFR1 serves as a general transcriptional regulator, as indicated by its association with the majority of active nuclear centers of RNA synthesis and processing, by the ability of nuclear FGFR1 to activate structurally distinct genes located on different chromosomes and by its stimulation of multi-gene programs for cell growth and differentiation. We propose that FGFR1 is part of a universal ''feed-forward-and-gate'' signaling module in which classical signaling cascades initiated by specific membrane receptors transmit signals to sequence specific transcription factors (ssTFs), while INFS elicited by the same stimuli feeds the signal forward to the common coactivator, CREB-binding protein (CBP). Activation of CBP by INFS, along with the activation of ssTFs by classical signaling cascades brings about coordinated responses from structurally different genes located at different genomic loci. J. Cell. Biochem. 90: 662–691, 2003.

Key words: FGF receptor; nuclear trafficking; gene regulation; development

Cells in the developing nervous system, and other tissues, move through distinct phases of proliferation, growth, migration, differentiation, and death during ontogeny. This process is guided by a plethora of signals emanating from growth factors, neurotransmitters, hormones, and extracellular matrix-associated molecules interacting with the cell surface. Cells are exposed to a variety of epigenetic factors at each phase. Some of these factors tend to direct the cells toward different, sometimes mutually exclusive, developmental states (Fig. 1). What remains ill defined is how signals generated by the plethora of extracellular factors (acting through specific receptors) and their intracellular signal transduction pathways may coordinately regulate structurally distinct genes at different genomic loci thereby enabling the execution of complex multi-gene developmental programs. This review discusses a novel signaling mechanism, INFS, that integrates

Grant sponsor: NSF; Grant number: IBN-9728923; Grant sponsor: NIH; Grant numbers: HL-49376, NS43621-01, GM23922.

*Correspondence to: Michal K. Stachowiak, Molecular and Structural Neurobiology and Gene Therapy Program, Department of Pathology and Anatomical Sciences, 317 Farber Hall, State University of New York at Buffalo, Buffalo, NY 14214. E-mail: mks4@buffalo.edu

Received 3 June 2003; Accepted 4 June 2003

DOI 10.1002/jcb.10606

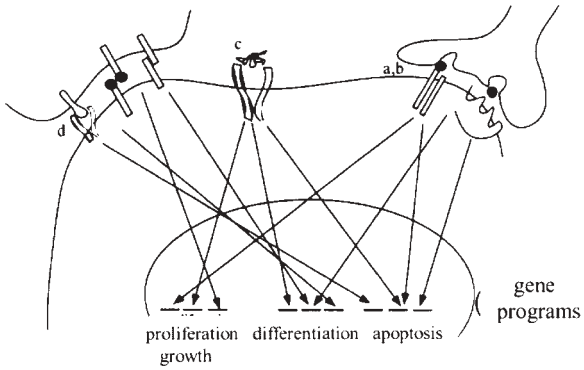

Fig. 1. At different developmental phases, cells are affected by a great variety of epigenetic factors including growth factors (c), neurotransmitters, hormones (a, b) and extracellular matrix-associated molecules (d) and their intracellular signaling pathways, which tend to direct the cells toward different, sometimes mutually exclusive, developmental states.

diverse, specific extracellular, and intracellular signals. This pathway induces coordinated responses of different genes, while allowing the specificity of signals to be preserved through specific classical signaling cascades and sequence specific transcriptional factors (ssTF).

FIBROBLAST GROWTH FACTORS (FGFS) AS INTRACRINE NUCLEAR SIGNALING FACTORS

The role of a membrane associated growth factor receptor is to transmit signals from the extracellular environment to the cytoplasm, according to the classical theory of signal transduction. Other cytoplasmic proteins and kinases serve as second messengers and propagate the signal downstream to the nucleus. However, not all growth factors produce their biological effects in this manner. In recent years, a number of laboratories have reported the presence of growth factors within the cell nucleus, suggesting that some of their biological effects are produced through direct interactions with nuclear effectors [Jans, 1994; Olsnes et al., 2003, reviews]. An important group of such factors are proteins encoded by the fibroblast growth factor-2 (*FGF-2*) gene. Evidence is accumulating that a variety of growth controlling agents and major intracellular signaling pathways, including Ca/PKC, cAMP, and tyrosine kinase initiated cascades, regulate cell proliferation, growth, differentiation, and functions by upregulating endogenous, nuclear FGF-2 [Puchacz et al., 1993; Ali et al., 1993; Delafontaine and Lou, 1993; Itoh et al., 1993; Stachowiak et al., 1994b; Joy et al., 1997; Fischer et al., 1997; Guo-Hong, 1998; Li and Yang, 1998; Moffett et al., 1998].

NUCLEAR LOCALIZATION OF HIGHER MOLECULAR WEIGHT (HMW) FGFS

FGFs consist of a large family of proteins (>21). Several members of the FGF family have a leader sequence (signal peptide), typical of secreted proteins, and act as extracellular growth factors (Fig. 2A). However, FGF-2 and a few other FGFs appear to have evolved to act inside the cells [Szebenyi and Fallon, 1999; Delrieu, 2000, reviews]. These proteins lack a signal peptide, but have acquired a nuclear localization signal (NLS), composed of positively charged amino acids [Abraham et al., 1986a,b; Jaye et al., 1986; Schweigerer et al., 1987; Vlodavsky et al., 1991; Moscatelli, 1988; Florkiewicz et al., 1991; Stachowiak et al., 1994b]. Translation of FGF-2 mRNA yields 18 kDa FGF-2 and additional higher molecular weight isoforms (HMW; 21–24 kDa in human or bovine cells [Florkiewicz et al., 1991; Stachowiak et al., 1994b; Joy et al., 1997] and 21.5–22 kDa in rat cells [Powell and Klagsbrun, 1991; Stachowiak et al., 2003]). All HMW FGF-2 contain a NLS, within their extended N-terminal sequence (Fig. 2A), which causes their nuclear accumulation (Fig. 2A,B) [Szebenyi and Fallon, 1999, review]. Thus, HMW FGF-2 isoforms are generally not found outside cells [Moscatelli, 1988; Florkiewicz et al., 1991; Stachowiak et al., 1994b; Bikfalvi et al., 1995; Joy et al., 1997; Peng et al., 2002]. FGF-2 (18 kDA), which lacks a NLS, is detected in the cytoplasm and the nuclear interior (Fig. 2B, left), probably because its small molecular size permits diffusion via nuclear pores [Renko et al., 1990; Powell and Klagsbrun, 1991; Stachowiak et al., 1994b; Peng et al., 2002]. Interestingly, in developing rat brain, both high and low molecular weight FGF-2 are found almost exclusively in the nuclear fraction (Fig. 2B, right) [Stachowiak et al., 2003]. Figure 2C illustrates the absence of FGF-2 from secretory vesicles and the plasma membrane and its extensive accumulation inside the nucleus of neuroendocrine bovine adrenal medullary cells (BAMC) [Peng et al., 2002]. In other cells, small amounts

of 18 kDa FGF-2 can be detected at the plasma membrane and outside the cell in the extracellular matrix [Moscatelli, 1988; Vlodavsky et al., 1991; Schechter, 1992]. This phenomenon could be a reflection of changes in plasma membrane integrity and thus signal cell injury. Many FGFs have leader sequences causing their secretion and subsequent production of biological effects by stimulating their plasma membrane associated receptors. Therefore, it is not surprising that FGF-2 can induce cellular responses when provided extracellularly, because it activates the same receptors as other members of the FGF family [Szebenyi and Fallon, 1999].

The importance of the intracellular localization of FGFs was established with studies that have shown: (i) FGF-2 does not need to be secreted in order to stimulate the proliferation of fibroblasts [Bikfalvi et al., 1995] or differentiation of avian Schwann cells [Sherman et al., 1993]; (ii) extracellularly added FGF-2 accumulates in the nucleus in a cell cycle-dependent manner [Bouche et al., 1987]; (iii) FGF-2 added to isolated nuclei stimulates rRNA synthesis [Bouche et al., 1987] and affects gene transcription in vitro [Nakanishi et al., 1992]; (iv) the mitogenic action of exogenous FGF-1 requires nuclear translocation of FGF1 [Imamura et al., 1990; Wiedlocha et al., 1994]; and (v) that nuclear FGF-2 stimulates cellular growth [Arese et al., 1999].

The interest in FGF-2 as a potential nuclear signaling molecule has increased greatly following the observation that its nuclear accumulation is a highly regulated process and correlates

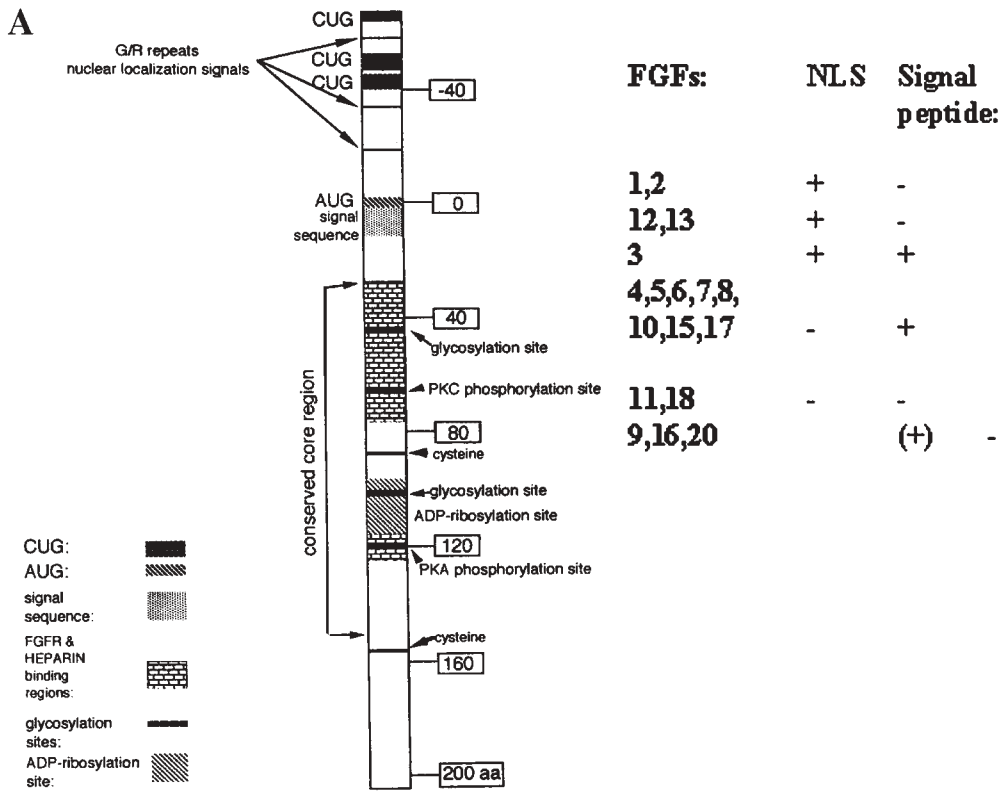

Fig. 2. Structure and subcellular localization of fibroblast growth factor-2 (FGF-2) and other FGFs. **A**: Structure of FGF-2 and list indicating the presence of signal peptide (SP) and nuclear localization signal (NLS)-like sequences in other FGFs (from Szebenyi and Fallon [1999]). **B**: Localization of FGF-2 isoforms in extranuclear (EN) and nuclear (N) fractions of TE671 cells transfected with a plasmid expressing all translational isoforms of human FGF-2 (left) [from Peng et al., 2002]. Subcellular localization of endogenous FGF-2 isoforms in the embryonic rat brain (E23) [Stachowiak et al., 2003]. **C**: Electron microscopic analysis of FGF-2-IR (20-nm immunogold particles) in BAMC. a, b: Enlarged cell areas. Cells were treated with 5-μM forskolin [from Peng et al., 2002]. No staining was detected when FGF-2 Ab was replaced with control IgG (not shown). The specificity of nuclear FGF-2 immunostaining was documented in several experiments in which FGF-2-IR either was abolished after the expression of FGF-2 was blocked with antisense FGF-2 oligonucleotides or appeared after transfection of FGF-2 into TE671 or glioma cells that express little or no endogenous FGF-2 [Stachowiak et al., 1994b; Joy et al., 1997; Moffett et al., 1996, 1998; Peng et al., 2002].

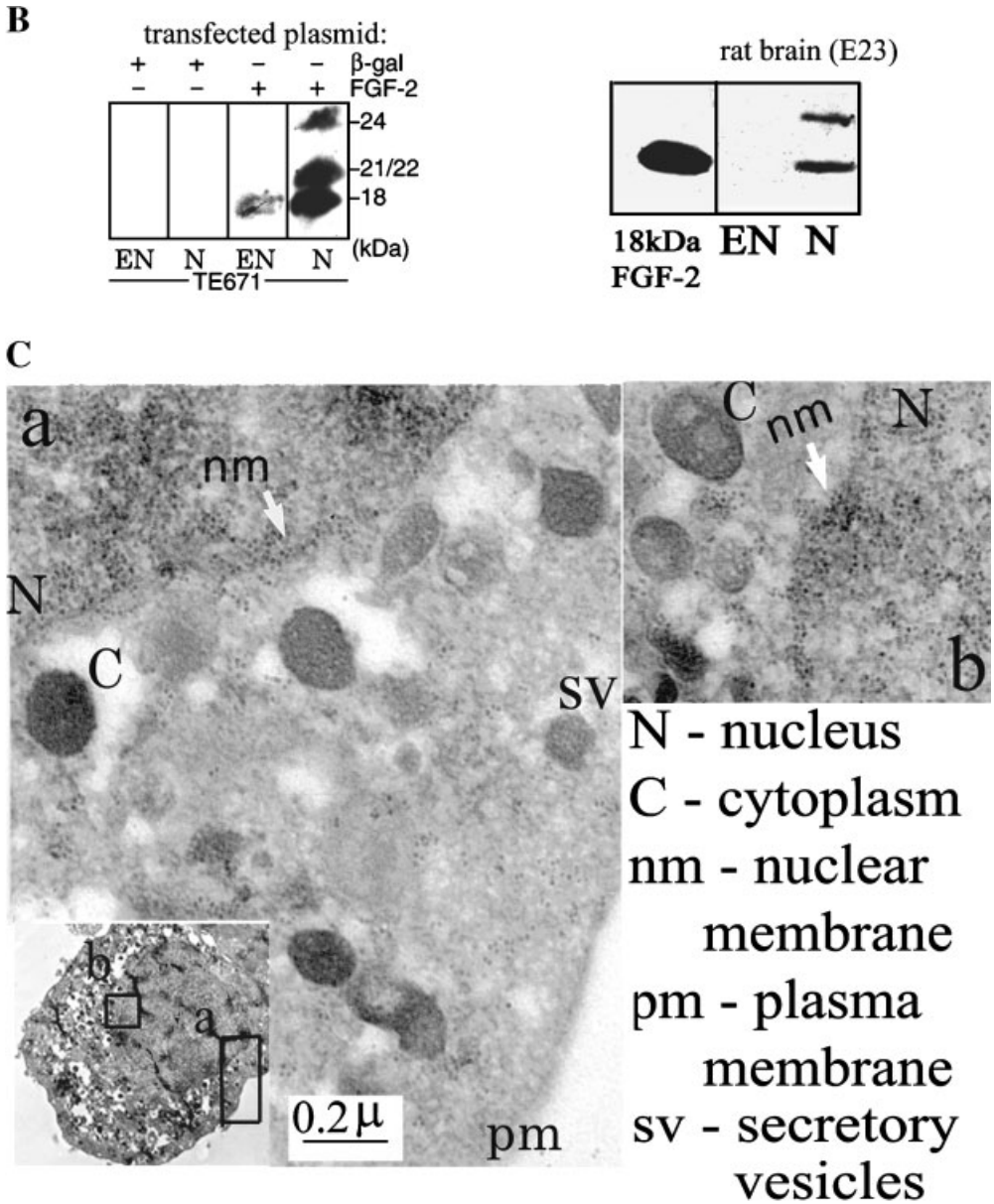

Fig. 2. (*Continued*)

with the transition of cells between major developmental stages and cell activity. Studies in our laboratory, and others, revealed an association between nuclear FGF-2 accumulation and (i) proliferation of glial cells; (ii) growth, differentiation, and functional activation of neurons; and (iii) activation of neuro-endocrine adrenal medullary cells by various extracellular factors and major intracellular signaling pathways [Stachowiak et al., 1994b, 1998; Joy et al., 1997; Peng et al., 2002; see also, Matsuda et al., 1992a,b; Woodward et al., 1992; Dono and Zeller, 1994; Klimaschewski et al., 1999; Clarke et al., 2001]. Thus, the concept of an "intracrine" growth factor-signaling molecule, translocated from the cytosol to the nucleus (without externalization), induces cellular responses has been proposed [Stachowiak et al., 1998]. The interest in FGF-2 as a nuclear signaling molecule further increased with the discovery of nuclear FGF receptors (discussed next).

DISCOVERY OF NUCLEAR FGFR

FGFs are known to interact with two main classes of receptors; high affinity tyrosine kinase

receptors which bind FGFs with picomolar affinity and are thought to mediate FGF cellular responses, and low affinity receptors which bind FGFs with nanomolar affinity and are characterized by the presence of heparan sulfate [Johnson and Williams, 1993; Wilkie et al., 1995]. Four genes encode the high affinity FGFRs with additional variants arising due to differential splicing. FGFR1–4 share a similar structure consisting of an N-terminal signal peptide, which directs FGFR synthesis to the endoplasmic reticulum (ER), two or three immunoglobulin-like domains which contain the ligand binding site, a single transmembrane domain, a split tyrosine kinase domain, and a C-terminal domain (Fig. 2A).

The presence of high affinity FGF-2 binding sites in the nuclei of BAMC, astrocytes, and glioma cells were found in an effort to determine the localization of FGFRs that could mediate the intracellular action of FGF-2 [Maher, 1996; Stachowiak et al., 1996a,b, 1997]. Astrocytes and BAMC were found to express only FGF receptor-1 (FGFR1), which accounts for the high affinity FGF-2 binding sites in the nucleus and cytoplasm. The number of high affinity FGF-2 nuclear binding sites was found to be greater than that on the cell surface in BAMC (Fig. 3F) [Stachowiak et al., 1996a,b, 1997]. In other types of cells (neurons, PC12 cells, or astrocytes), FGFR-IR in the nucleus was similar to or less intense than in the cytoplasm [Maher, 1996; Stachowiak et al., 1996b, 2003]. Localization of FGFR1 within the nuclei was also observed in the rat brain [Gonzalez et al., 1995; Clarke et al., 2001; Stachowiak et al., 2003], PC12 cells, fibroblasts, endothelial cells [Maher, 1996; Reilly and Maher, 2001], and in cochleovestibular ganglion cells [Bilak et al., 2003]. Figure 3B illustrates the relative distribution of FGFR1 between the nuclear and extranuclear fractions of the rat brain.

Nuclear FGFR1 (nFGFR1) is full length (Fig. 3C), undergoes autophosphorylation, and phosphorylates other proteins in a FGF-2 stimulated manner [Stachowiak et al., 1996a,b]. Analysis of nFGFR1 revealed the presence of different degrees of glycosylation (Fig. 3B–D), corresponding to the following sizes: 103, 118, and 145 kDa [Stachowiak et al., 1998]. The *FGFR1* gene lacks potential splicing sites that could result in the splicing out of the sequences encoding either the signal peptide (leader sequence) or the transmembrane domain [Hou et al., 1991]. Furthermore, transfection of cells with a cDNA encoding full length FGFR1 leads to the accumulation of recombinant receptor in the extranuclear fraction (representing mainly membrane associated FGFR1) as well as in the nucleus (Fig. 3D) indicating that an unusual processing of FGFR1 RNA is neither likely nor necessary to generate the nuclear form of the receptor [Stachowiak et al., 1997; Peng et al., 2001, 2002].

Immunohistochemistry in combination with confocal microscopy confirmed these observations revealing a distinct intranuclear and membrane presence of FGFR1-immunoreactivity (IR) (Fig. 3F) [Maher, 1996; Stachowiak et al., 1996a,b, 1997, 2003; Peng et al., 2001, 2002;

Fig. 3. Structure and subcellular localization of fibroblast growth factor receptor-1 (FGFR1). **A**: General structure of FGFR1 and the receptor mutants used in our studies. The domains recognized by the FGFR1 antibodies (C-term and McAb6) used in our studies are indicated. **B**: Distribution of FGFR1 in nuclear (N) and extranuclear (EN) fractions of the embryonic rat brain (in the same fractions, we also analyzed FGF-2; Fig. 1B, right) [from Stachowiak et al., 2003]. **C**: Nuclear FGFR1 contains both C-terminal (intracellular) and N-terminal (extracellular, ligand-binding) domains. Nuclear extracts of BAMC were immunoprecipitated with the C-term FGFR1 Ab and the immunoprecipitates subjected to Western blotting with McAb6 against the extracellular domain of FGFR. Total cell extract (input), immunoprecipitate (ppt), and the proteins in the remaining supernatant (sup) were subjected to Western immunoblotting [from Stachowiak et al., 1996a]. **D**: Nuclear and extranuclear localization of recombinant FGFR1 or FGFR1-EGFP in transfected TE671 cells which express low levels of endogenous receptor [from Peng et al., 2002]. **E**: Nuclear FGFR1 is not derived from the plasma membrane. Cell surface proteins in FGFR1 transfected TE-671 cells were biotinylated with cell impermeable NHS-sulfobiotin and biotinylated receptor was detected only in the extranuclear [Peng et al., 2002]. These data also indicated that the appearance of FGFR1 in the nucleus was not the result of contamination by plasma or cytoplasmic membrane-associated FGFR1 (also documented using other subcellular marker proteins [Stachowiak et al., 1996a,b]). **F**: FGFR1 is localized in the nuclear interior. BAMC were labeled with the C-term FGFR1 Ab (**Panels I** and **II**) or N-terminal FGFR1 McAb6 (**Panel IV**) [Hanneken et al., 1995] or without primary Ab (**Panel III**). FGFR1 immunoreactivity was abolished by preincubating the C-term FGFR1 Ab with its cognate peptide (II). **Panel V** shows consecutive confocal sections through the nuclei illustrating the presence of FGFR1 in the nuclear interior. The specificity of the nuclear staining with the FGFR antibodies was further documented in experiments in which FGFR1-IR appeared after transfection of TE671 or SF761 glioma cells that express only very low levels of endogenous receptor with a plasmid expressing FGFR1 [Stachowiak et al., 1998, 2003; Peng et al., 2001, 2002; Reilly and Maher, 2001].

Bilak et al., 2003]. Nuclear FGFR1 has been detected with an array of different FGFR1 antibodies (Fig. 3F) and with antibodies against different tags, when transfected recombinant FGFR1 was examined. [Stachowiak et al., 1996a,b, 1997; Peng et al., 2002; our unpublished observations]. The pattern of immunofluorescence observed in consecutive confocal sections through cells showed that the nuclear interior contained FGFR1 immunofluorescence (Fig. 3F, part V) [Maher, 1996; Stachowiak et al., 1996a,b]. Translocation of FGFR1 through the nuclear membrane and subsequent nuclear accumulation was also demonstrated by immuno-electron microscopy [Stachowiak et al., 1996a]. Thus, the nuclear interior is a

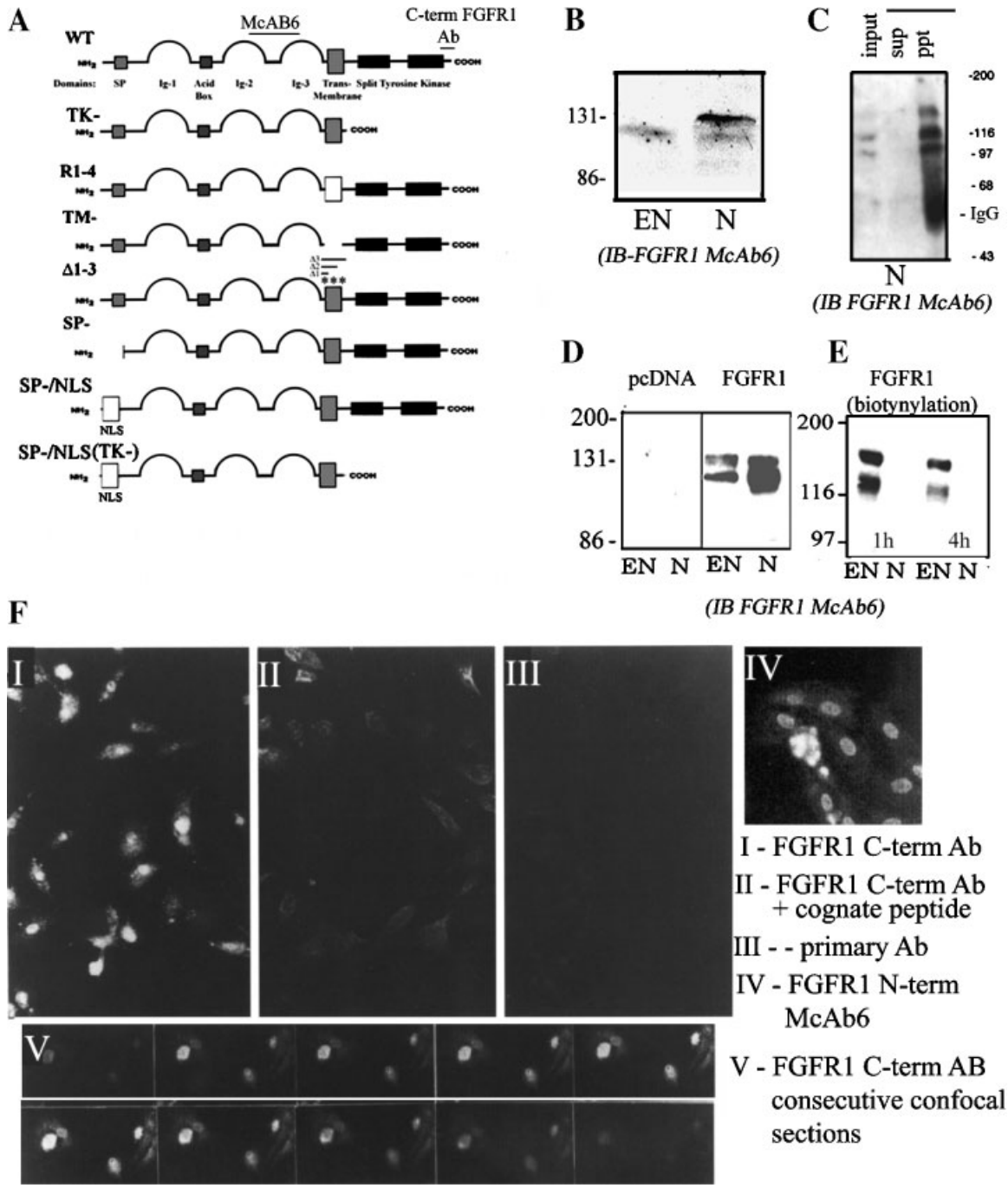

Fig. 3.

major subcellular site of functional FGFR1 in vitro and in vivo.

SOURCE OF NUCLEAR FGFR1

FGFR1 signal peptide causes constitutive fusion with the plasma membrane; therefore, the mechanism of nuclear entry was unclear. Although incubation of fibroblasts with exogenous 18 kDa FGF-2 resulted in the nuclear translocation of FGFR1 [Maher, 1996], in glioma cells, BAMC, or TE671 medulloblastoma cells, the nuclear accumulation of FGFR1 could not be induced by extracellular FGF-2. Instead, nuclear accumulation of FGFR1 was elicited by the same stimuli that induced the nuclear accumulation of the HMW FGF-2, including growth factors, hormones, and the stimulation of cAMP and Ca^{+2}/PKC second messengers [Stachowiak et al., 1994b, 1996a,b; Peng et al., 2002]. Nuclear FGFR1 was shown not to be derived from the cell surface by NHS-sulfobiotin incubation with glioma and TE671 medulloblastoma cells did not lead to an appearance of biotinylated endogenous or transfected receptor in the nucleus [Stachowiak et al., 1998; Peng et al., 2002]. However, glycosylation of nFGFR1 indicated that this protein is processed, at least partially, through the ER-golgi. Hence, we had hypothesized that the association of FGFR1 with ER membranes may not be stable leading to the receptor being released into the cytosol before the endoplasmic vesicles fuse with the plasma membrane.

To test this hypothesis, subcellular trafficking of FGFR1 fused to enhanced green fluorescent protein (EGFP) in live cells was investigated. The biological functions and subcellular distribution of FGFR1 and its mutants were not affected by fusion to EGFP [Peng et al., 2001, 2002; Myers et al., 2003]. Subcellular localization of EGFP fluorescence was examined using quantitative confocal imaging and confirmed by biochemical fractionations and Western immunoblotting [Myers et al., 2003]. Transfected FGFR1-EGFP initially accumulated in cytoplasmic, golgi-ER-like vesicles (Fig. 4A) and then in the plasma and nuclear membranes and thin filopodia, similar to newly synthesized endogenous FGFR1 [Stachowiak et al., 1996a,b; Peng et al., 2001, 2002]. However, FGFR1-EGFP fluorescence was not observed in non-membrane cytosolic regions, outside vesicles, or within the nuclear interior. Gradually, the FGFR1-EGFP content decreased within golgi-ER vesicles, increased in the cytosol, and accumulated in the nucleus (excluding the nucleoli) (Fig. 4A). Nuclear entry of FGFR1-EGFP was observed in rapidly proliferating human TE671 cells, in slowly proliferating human astrocytes, post-mitotic (BAMC) (Fig. 4A), and differentiating human neurons (Fig. 7B). Thus, nuclear accumulation of FGFR1 is independent of karyokinessis. This sequence of FGFR1-EGFP localization was consistent with the concept that proteins containing signal peptides are destined to constitutively associate with membranes. In addition, it suggested that FGFR1-EGFP is released into the cytosol from cytoplasmic membranes prior to its accumulation in the nucleus [Myers et al., 2003].

Single transmembrane (TM) proteins are anchored in the membrane by a hydrophobic, alpha-helical TM domain that interacts with the lipid bilayer. The Garnier algorithm predicted a typical alpha-helical structure for the FGFR4 TM domain and FGFR2 and FGFR3 both have beta-sheet regions followed by shorter alpha-helices. However, the TM domain of FGFR1 has a beta-sheet-turn-beta-sheet structure with no predicted alpha-helix [Myers et al., 2003]. Furthermore, the FGFR1 TM domain has four hydrophilic amino acids that divide it into short hydrophobic peptides. Similar hydrophilic interruptions exist in the TM domain of T-cell receptor-α [Shin et al., 1993], which causes its release from the ER membrane [Yang et al., 1998]. In contrast, FGFR2–4 have TM domains with undisrupted hydrophobic chains. We have constructed a series of FGFR1 mutants (Fig. 3A) designed to examine the significance of the TM domain in the nuclear accumulation of FGFR1. Replacement of the FGFR1 TM domain with the TM domain of FGFR4 hindered the release of the chimericFGFR1/R4-EGFP receptor into the cytosol. FGFR1/R4 was associated with the ER-golgi and with the nuclear membrane but was absent from the cytosol and the nuclear interior. Mutants were constructed to increase the number of polar TM amino acids in FGFR1, FGFR1(Δ1)(V391R), FGFR1(Δ2)(V391R and C381R) and FGFR1(Δ3)(V391R, C381R and L386D). Increasing the polarity of FGFR1 resulted in a decrease in the nuclear and intracytoplasmic (golgi-ER) membrane receptor content and an increase in cytosolic and intranuclear contents [Myers et al., 2003; Fig. 4B,C]. Shifting of FGFR1 from golgi-ER and mem-

branes to the cytosol with increasing TM domain polarity and the opposite change when the TM domain of FGFR1 was replaced with that of FGFR4 (see Fig. 4B,C) was confirmed by Western blotting of cell fractions [Myers et al., 2003]. The concentrations of recombinant FGFR1 proteins in the nuclear and cytosolic compartments showed a significant correlation ($r = 0.89 + 0.19$) (Fig. 4D) indicating that the cytosolic and nuclear receptor pools are in

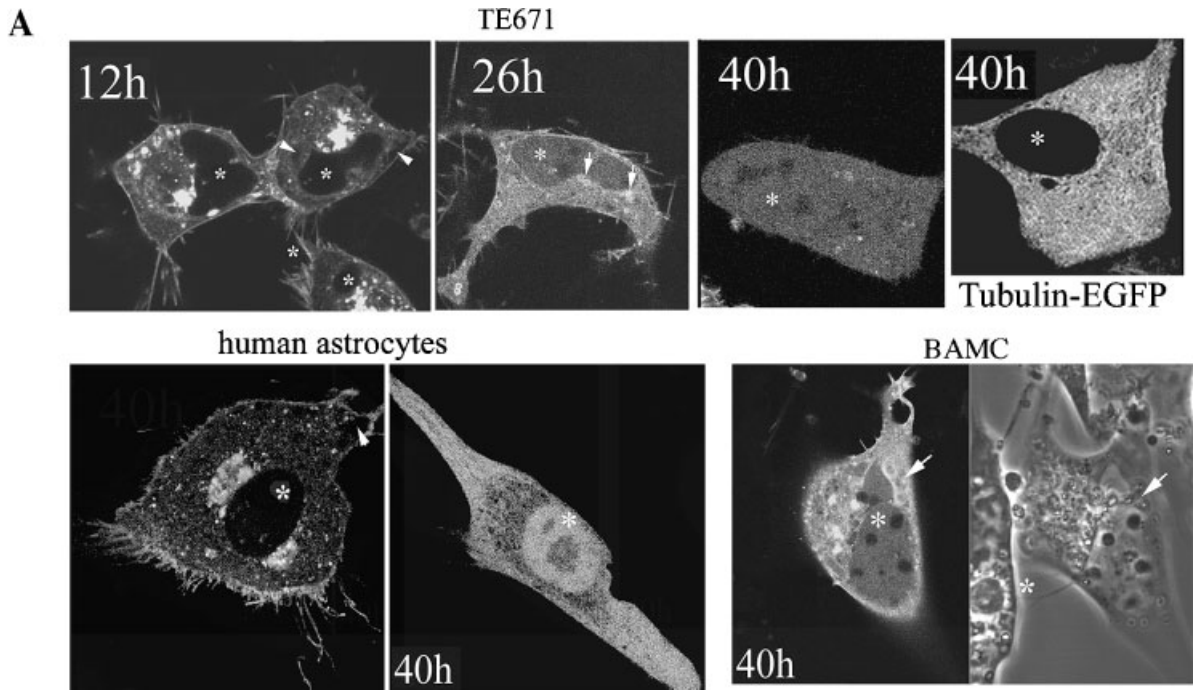

Fig. 4. Source of nuclear fibroblast growth factor receptor-1 (FGFR1). **A**: FGFR1-EGFP is present in the cell nucleus independent of mitotic activity. All panels except one (tubulin-EGFP) show FGFR1-EGFP images. TE671 medulloblastoma cells (doubling time: approximately 12 h)—subcellular trafficking of FGFR1-EGFP at 12, 26, and 40 h post-transfection [from Peng et al., 2002; Myers et al., 2003]. Human astrocytes (doubling time: 3–4 days)—examples of two types of FGFR1-EGFP localization are shown: (i) left, membrane and golgi-ER associated, non-nuclear localization; and (ii) right, cytosolic-nuclear localization (E.K. Stachowiak and M.K. Stachowiak, unpublished results). Bovine adrenal medullary cells (BAMC; postmitotic cells)—40 h after FGFR1-EGFP transfection [Myers et al., 2003]. Mid-nuclear confocal sections of live cells are shown. Arrowheads indicate cytosolic regions devoid of fluorescence; arrows indicate golgi-ER vesicles; asterisks indicate nucleus. **B**: Quantitative analysis of receptor distribution. Mutations in the transmembrane domain or the signal peptide affect the subcellular localization of FGFR1-EGFP (for FGFR1 mutants see Fig. 3A). FGFR1-EGFP fluorescence associated with the plasma membrane, perinuclear golgi-ER vesicles, nuclear interior, and in the peripheral cytoplasm (cytosol) was measured using confocal microscopy and NIH's Image V1.23q software [Myers et al., 2003]. To account for the different overall levels of FGFR1-EGFP in different cells and for photobleaching, the relative fluorescence intensity (RFI) of each compartment was calculated by dividing the mean fluorescence intensity by the mean fluorescence intensity of the whole cell within the analyzed confocal section after background (non-cellular) pixel intensity was subtracted. RFI-multivariate analysis: overall differences between FGFR1 constructs, differences between subcellular compartments and the interaction between these two variables were all significant at $P < 0.001$. Posthoc LSD; */** different from WT ($P < 0.05, < 0.001$); +, ++ different from TM ($P < 0.05, < 0.001$) [from Myers et al., 2003]. **C**: Partition of wild type and mutant FGFR1-EGFP between the cytosol and golgi-ER compartments. Bars represent mean ± SEM ratios calculated for the individual cells. ANOVA: Overall effects of FGFR1 mutations on cytosol/nuclear membrane and cytosol/golgi ER $P < 0.001$ [from Myers et al., 2003]. **D**: Correlation between FGFR1-EGFP distribution in the intranuclear and cytosolic compartments. Cells transfected with wild type FGFR1, FGFR1/R4, or individual FGFR1Δ1,2, 3 mutants fused with EGFP. Nuclear and cytosolic RFIs were estimated for each cell and their values were subjected to linear regression analysis; $r = 0.89 \pm 0.19$ [from Myers et al., 2003]. **E**: Hypothetical mechanism for the release of FGFR1 from the ER membrane. During translation of membrane-inserted proteins such as FGFR1, the signal peptide binds to the pore complex and the polypeptide elongates and translocates into the ER lumen. When the transmembrane domain (stop-transfer signal) is reached, the protein can either dissociate from the translocon complex and anchor itself firmly in the lipid bilayer or can be transported back into cytosol (protein-conducting channel formed by Sec61 complex is responsible for both forward (insertion) and retrograde (removal) of proteins across the ER membrane [Matlack et al., 1998; Romisch, 1999]). The retrograde transport is coupled to the 26S proteasome and the cytosolic FGFR1 may be degraded or rapidly transported to the nucleus via importin-B mediated manner. Atypical TM domain appears to be responsible for the release of FGFR1 from the ER membranes [Myers et al., 2003].

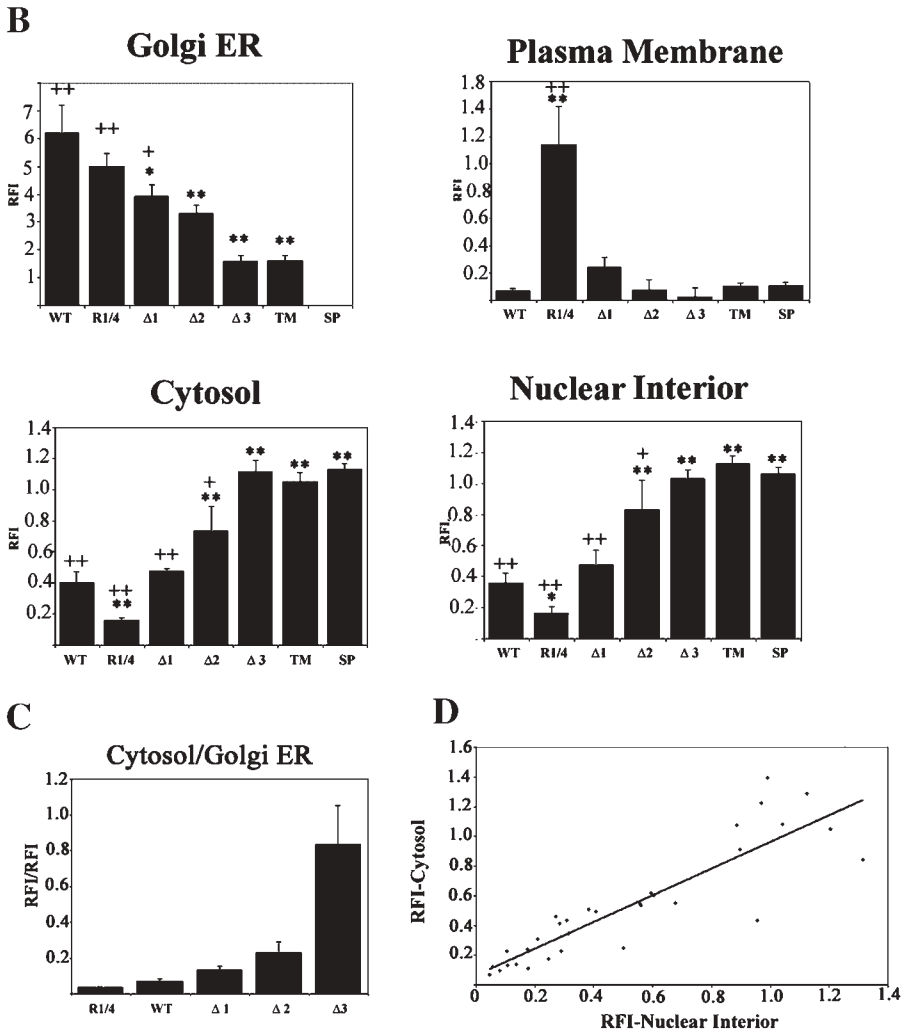

Fig. 4. (*Continued*)

equilibrium. A resident, soluble, cytosolic receptor (FGFR1(SP-)-EGFP) effectively entered the nucleus. FGFR1(SP-)-EGFP is a mutant that lacks the signal peptide of the FGFR1, which results in the translation of receptor mRNA and cytosolic accumulation of the protein without the initial insertion into the ER membrane [Myers et al., 2003]. These experiments have shown that the atypical TM structure appears to be critical for the nuclear trafficking of FGFR1.

We propose that FGFR1 may enter the nucleus through retrograde transport. Retrograde transport (golgi to cytosol) has been shown to occur and can be viewed as a reversal of the process of the integration of membrane proteins into the ER membrane [Matlack et al., 1998; Romisch, 1999]. Proteins structurally similar to FGFR1 (e.g., MHC class I molecules [Wiertz et al., 1996], mutant insulin receptor [Imamura et al., 1998], or T-cell receptor-α [Yang et al., 1998]) are retrogradely transported from the ER membrane into the cytosol. Retrograde protein transport from the ER membrane back into the cytosol is coupled to the 26S proteasome, thereby resulting in the degradation of the "membrane" protein in the cytosol [Shin et al., 1993; Imamura et al., 1998]. We found an increase in the cytosolic content of FGFR1 in cells treated with lactacystin [Myers et al., 2003], a specific proteasome inhibitor, suggesting that FGFR1 in the ER membrane can undergo a similar type of processing, that is, release from the ER membrane back into the cytosol, where it may be degraded or rapidly transported to the nucleus (Fig. 4E).

FGFR1 was shown to be transported into the nucleus in association with importin-β, a critical component of multiple nuclear import pathway

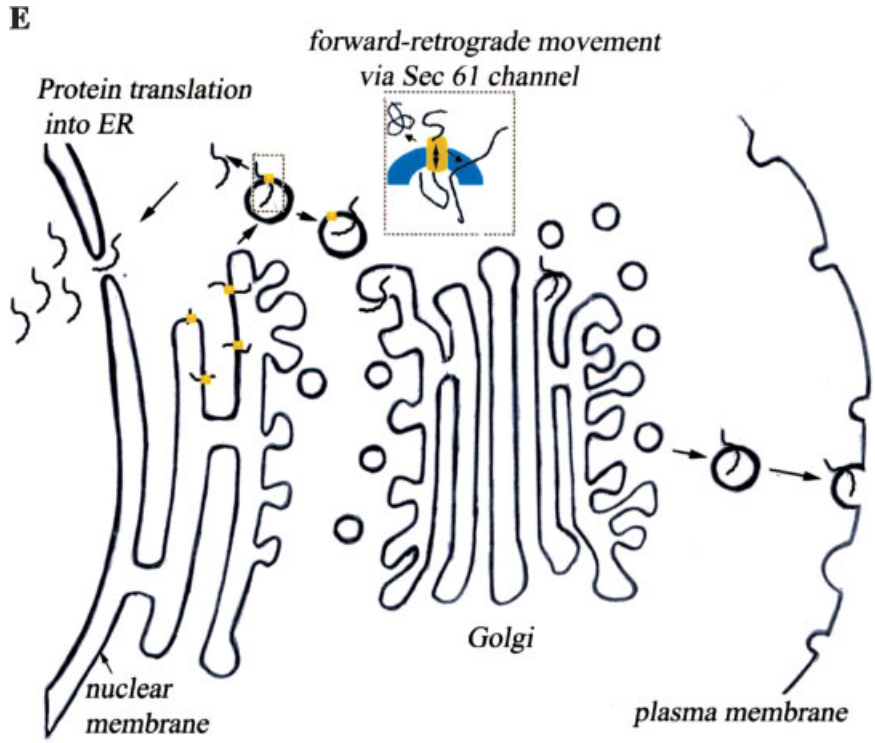

Fig. 4. (*Continued*)

(Fig. 5; Reilly and Maher, 2001). Importin-β is a soluble protein which trafficks between the cytoplasm and the nucleus, therefore the interaction with FGFR1 must occur in the cytoplasm. Importin-β can carry cargo to the nucleus either directly or in conjunction with an adapter protein. Preliminary data suggest that FGFR1 does not interact directly with importin-β, indicating that an adapter protein probably mediates the interaction. Most adapter proteins interact with the NLS of proteins destined for the nucleus. Since FGFR1 lacks a typical NLS, it is likely to be transported into the nucleus in association with another NLS-containing protein(s). A candidate protein for the transport is HMW FGF-2, which contains a NLS. Consistent with this idea, FGF-2 appears to enter the nucleus along with FGFR1, in all experiments in which this was examined.

NUCLEAR FGFR1 REGULATES CELL PROLIFERATION, GROWTH, AND OTHER FUNCTIONS

INFS Regulates the Proliferation of Glial Cells

Quiescent astrocytes demonstrate cytoplasmic FGF-2 immunoreactivity (FGF-2-IR), but lack nuclear FGF-2 staining in normal human brain tissue. In contrast, prominent FGF-2 immunostaining in the nuclei of reactive astrocytes was found in brain areas with extensive neuronal loss and gliosis. Additionally, FGF-2 accumulated nearly exclusively in the nuclei of transformed glia within *glioblastoma* tumors (FGF-2 presence was not detected outside of cells). These data suggested that nuclear FGFR1/FGF-2 play a role in regulating the proliferation of glial cells [Joy et al., 1997].

An association between the nuclear accumulation of endogenous FGF-2 and FGFR1 and cell proliferation was confirmed using cultured human astrocytes [Joy et al., 1997; Stachowiak et al., 1996b, 1997, 1998]. Astrocyte proliferation could be triggered by cell contact inhibition release and further enhanced by treatment with serum, EGF, PDGF, angiotensin II (AII), and by the direct stimulation of adenylate cyclase with forskolin or PKC with PMA. The same treatments induced the intranuclear accumulation of FGFR1 and FGF-2 (Fig. 6A–C), which was accompanied by an overall increase in nuclear FGF-2-inducible tyrosine kinase activity [Joy et al., 1997; Moffett et al., 1996; Stachowiak et al., 1996b, 1997]. Additionally, the nuclear

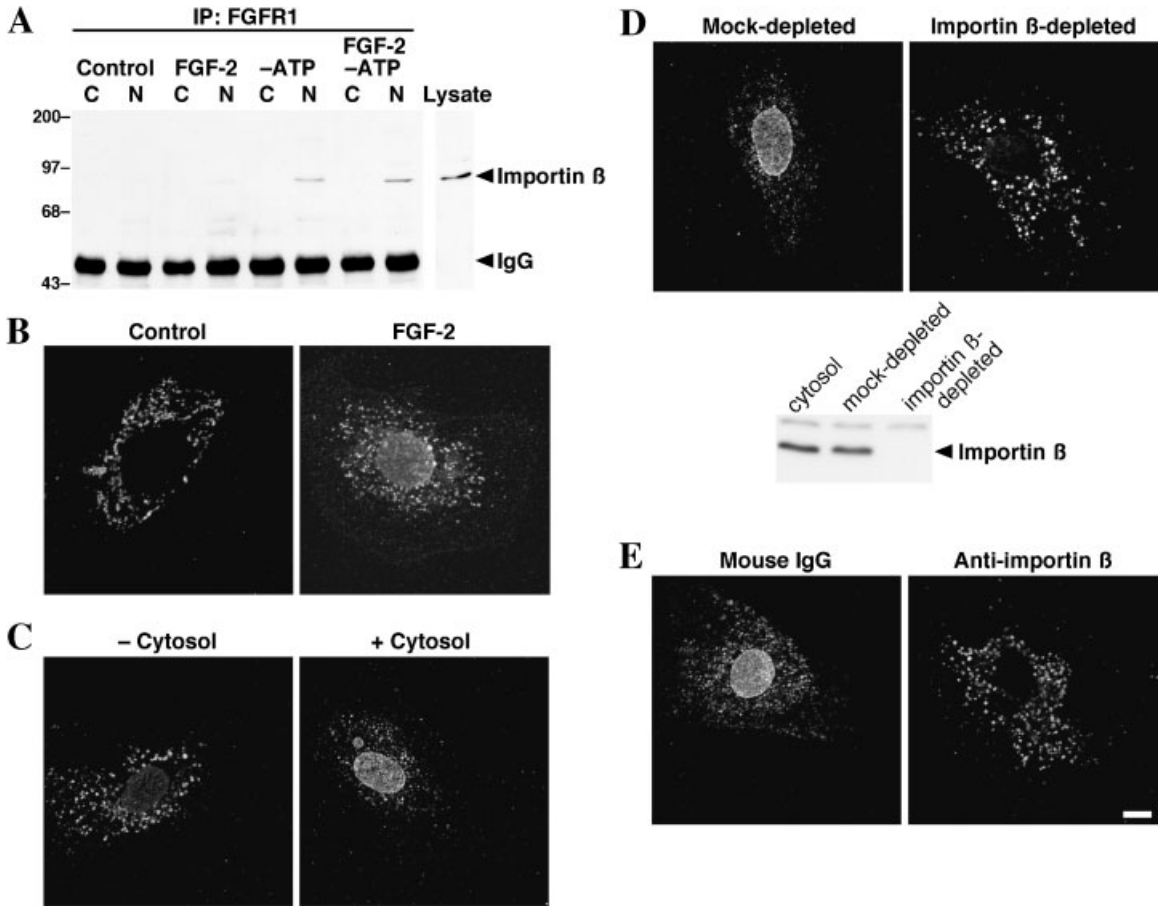

Fig. 5. Transport of fibroblast growth factor receptor-1 (FGFR1) to the nucleus is mediated by importin-β [from Reilly and Maher, 2001]. **A**: Co-immunoprecipitation of importin-β with FGFR1. Cells were untreated or subjected to ATP depletion by treatment with oligomycin B and 2-deoxyglucose for 2 h, in the absence or presence of FGF-2, and separated into cytosolic (C) and nuclear (N) fractions. FGFR1 was immunoprecipitated from equal amounts of protein, and complexes were separated by SDS–PAGE and immunoblotted for importin-β. The whole cell lysate is shown for comparison, the immunoglobulin heavy chain (IgG) is indicated, and molecular weights are indicated in kilodalton. **B–E**: Nuclear translocation of FGFR1 in Swiss 3T3 fibroblasts was examined using an in vitro nuclear import assay [Adam et al., 1990], and cells were analyzed by FGFR1 immunostaining and confocal microscopy. Unpermeabilized cells (b) were untreated or incubated for 30 min with FGF-2. Digitonin-permeabilized cells (c–e) were incubated for 30 min with FGF-2 in the absence or presence of exogenous cytosol (c), with mock-depleted or importin-β-depleted exogenous cytosol (d), or with exogenous cytosol plus mouse IgG or a neutralizing antibody against importin-β (e). Depletion of importin-β from exogenous cytosol was confirmed by immunoblotting (d). Bar, 10 μm.

accumulation of FGFR1 in human astrocytes, released from contact inhibition, occurs in parallel with the induction of the late G1-phase cyclin D1 (Fig. 6B).

The oscillations in the nuclear content of endogenous FGF-2 and FGFR1 which accompanied the changes in astrocyte proliferation suggested that FGF-2/FGFR1 might control cell proliferation by acting directly in the nucleus. In support of this idea, we found that proliferation of glioma cell lines is stimulated by intracellularly expressed FGF-2, but not by exogenous FGF-2 [Joy et al., 1997]. Stimulation of cell proliferation was achieved by the stable transfection of the glioma cells with either 18 kDa FGF-2 or with HMW-FGF-2 isoforms [Fig. 6D; Joy et al., 1997], which do not associate with the plasma membrane and are not found outside the cells [Florkiewicz et al., 1991; Bikfalvi et al., 1995]. Furthermore, the increase in cell proliferation was resistant to treatment with myo-inositol hexakis [dihydrogen phosphate] (IP6) [Joy et al., 1997]. IP6 is an antagonist, which prevents the interaction of FGF-2 with cell surface FGFR, but does not inhibit the effects of intracellular FGF-2 [Sherman et al., 1993; Morrison et al., 1994; Peng et al., 2002].

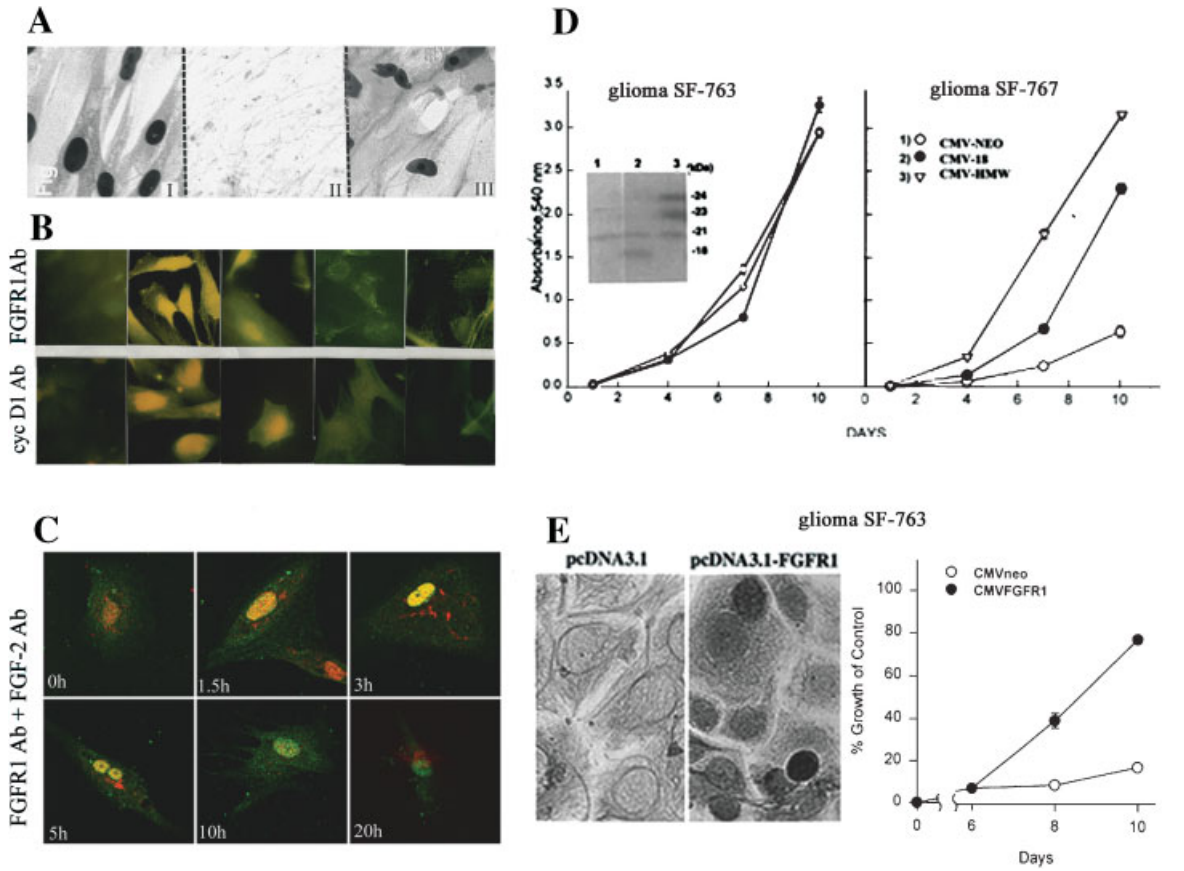

Fig. 6. In glial cells, the nuclear accumulation of fibroblast growth factor-2 (FGF-2) and FGFR1 accompanies entry into the cell cycle and stimulates cell proliferation. **A**: Cell-density dependent expression of nuclear FGF-2 in cultured human astrocytes [Joy et al., 1997]. In subconfluent cultures, proliferating cells express high levels of FGF-2 predominantly in the cell nucleus (I). Upon reaching the confluent state, the expression of nuclear FGF-2 is turned off (II) but can be reinstated by locally reducing the cell density (scraping the culture dish) (III) [from Joy et al., 1997]. **B**: FGFR1 accumulates transiently in the nuclei of astrocytes released from cell contact inhibition. Induction of late G1 phase cyclin D1 follows a similar time course [DNA replication (S-phase)] occurred after the nuclear FGFR1 content and cyclin D1 levels were reduced between 10 and 20 h after the release from contact inhibition [not shown, Stachowiak et al., 1997]. **C**: In subconfluent cultures, serum-starved astrocytes enter the cell cycle upon stimulation with 10% fetal calf serum in concert with the rapid and transient co-accumulation of FGFR1 (red) and FGF2 (green) in the same nuclei (colocalized pixels are shown in yellow color). **D**: Stable transfection of SF763 glioma cells with nuclear HMW forms of FGF-2 stimulates cell proliferation [Joy et al., 1997]. **E**: Stable transfection of SF763 glioma cells with FGFR1 increases cell proliferation (transfected FGFR1 accumulates predominantly in the cell nucleus [Stachowiak et al., 1997]).

To specifically address the role of nuclear FGFR1 in cell proliferation, stable transfection of FGFR1 into SF-763 glioma cells, which do not express FGFR1, was performed. FGFR1 was found intracellularly in transfected cells, localized almost exclusively in the nucleus [Stachowiak et al., 1997, 1998]. Expression of nuclear FGFR1 was accompanied by an increase in spontaneous cell proliferation in the absence of exogenously added FGF-2 (Fig. 6E). This stimulation was resistant to the treatment with FGFR antagonists that block the surface receptors and to additional stimulation with exogenous FGF-2. These results indicate that the increase in cell proliferation occurred independently of cell surface FGFR1 and could be dependent on nuclear FGFR1.

The accelerated proliferation of glioma cells expressing HMW FGF-2 or FGFR1 represented an increase in the mitotic activity of individual cells. Flow cytometry revealed a threefold increase in cells residing in the S and G2/M phases in cultures transfected with FGFR1 or HMW FGF-2 compared to a 1.7-fold increase in cells expressing control plasmids [Joy et al., 1997; Stachowiak et al., 1997]. This finding, along with the nuclear accumulation of endogenous FGF-2 and FGFR1 in glial cells in the late G1

phase, indicated that nuclear FGF-2 and FGFR1 stimulate cells to enter the S-phase of the cell cycle. Furthermore, later studies [Reilly and Maher, 2001] demonstrated that the activities of genes controlling cell proliferation could be stimulated by nuclear FGFR1.

Neoplastic glioma cells proliferate continuously, unresponsive to cell-contact inhibition, which is in contrast to the transient burst of controlled growth experienced by reactive astrocytes in gliotic tissue. The constitutive growth of glioma cells was also observed in vitro and was associated with constitutively high levels of nuclear FGF-2 and FGFR1 [Moffett et al., 1996; Stachowiak et al., 1996b, 1997]. These studies suggest that cell density-dependent regulation of nuclear FGF-2 and FGFR1 serves to control cell proliferation in normal glial cells. However, in neoplastic glioma cells, the constitutive presence of FGF-2 and FGFR1 in the nuclei could promote proliferation that is insensitive to cell–cell contact inhibition.

INFS MEDIATES CAMP AND BONE MORPHOGENETIC PROTEIN-7 (BMP-7) INDUCED NEURONAL DIFFERENTIATION

FGF-2 accumulates in the nuclei of cerebellar cortical neurons as they extend their axons toward the deep cerebellar nuclei in the developing rat brain. Once synaptic connections are established and axonal growth ceases, the nuclear expression of FGF-2 is turned off [Matsuda et al., 1992a]. Consistent with this early observation, we found that during neurogenesis in the rat brain (embryonic day 23), FGF-2 is located exclusively in the nucleus, while FGFR1 is found predominantly in the nucleus (see Fig. 2B, right; Fig. 3B). In contrast, in the adult brain, FGF-2 was observed in both nuclear and non-nuclear fractions [Stachowiak et al., 2003].

Stimuli that promote neuronal-like differentiation in postmitotic adrenal medullary cells (e.g., depolarization with vertradine, stimulation of adenylate cyclase with forskolin) and dendritic outgrowth in rat sympathetic neurons in vitro (e.g., treatment with BMP-7) induces the nuclear accumulation of FGFR1 and FGF-2 [Stachowiak et al., 1994b, 1996b, 1998; Peng et al., 2001; Horbinski et al., 2002]. We have used cultures of human neuronal progenitor cells (HNPC) as a model to study the role of INFS in neuronal differentiation [Piper et al., 2001; Stachowiak et al., 2003]. Differentiation of HNPC involves exit from the cell cycle, outgrowth of dendrites and axons, and expression of neuron-specific cytoskeletal, synaptic, and ion channel proteins. In the presence of FGF-2/EGF, HNPC proliferate and display characteristics of undifferentiated cells with short processes and an absence of voltage dependent K^+ or Na^+ currents. When HNPC are treated with dBcAMP, forskolin, or BMP-7, they exit the cell cycle, grow long neuritis, and express neuron-specific β-III Tubulin, MAP2, Nf-L, α-internexin, glutamate receptors, and voltage-dependent and neurotransmittter receptor-regulated ionic currents.

To explore the role of INFS in neuronal differentiation, HNPC were transfected with FGFR1-EGFP which resulted in the accumulation of FGFR1 in the cytoplasm as well as the nucleus of live cells (Fig. 7B) and induced the growth of long processes in the absence of dBcAMP treatment (Fig. 7C) [Stachowiak et al., 2003]. Furthermore, HNPC transfected with a mutant FGFR1 (FGFR1(SP-/NLS) in which the signal peptide (SP) was replaced with a NLS (see Fig. 7B) so as to direct it specifically to the nucleus also showed the growth of long processes (Fig. 7C) (for the structures of all FGFR1 mutants described in this study, see Fig 3A). In contrast, FGFR1/R4, which was membrane associated did not enter the nucleus (Fig 7B), and failed to induce differentiation (Fig. 7C). In all cases, the transfected receptors were expressed at levels similar to those of endogenous FGFR1 thus excluding the possibility that abnormal overexpression may be responsible for the differentiation effect [Stachowiak et al., 2003].

Analysis of the expression of intermediate filament proteins was then undertaken to confirm that HNPC transfected with different FGFR1 constructs truly undergo neuronal differentiation. Non-differentiated neuroepithelial cells express vimentin and nestin, which are replaced by α-internexin in cells committed to a neuronal lineage (neuronal progenitors), but not glial lineage. More mature neurons express the neurofilament triplet subunits: Nf-L, Nf-M, and Nf-H, which either replace or are expressed with α-internexin [reviewed in Evans et al., 2002]. Figure 7D shows representative staining of HNPC transfected with wild type FGFR1. All cells exhibited strong staining with the α-internexin antibody demonstrating

their neuronal lineage [Fig. 7D; Stachowiak et al., 2003]. The pattern of Nf-L expression in vector-transfected cells (weak perinuclear staining) and in FGFR1/EGFP- or FGFR1 (SP-/NLS)/EGFP-transfected cells (increased perinuclear and cytoplasmic Nf-L and strong neuritic Nf-L immunoreactivity) was essentially the same as in proliferating and dB-cAMP-differentiated HNPC, respectively [Stachowiak et al., 2003]. Little or no neuron-specific enolase (NSE) expression was detected in proliferating, vector-transfected cells, while strong NSE immunofluorescence was seen in FGFR1/EGFP- and FGFR1(SP-/NLS)/EGFP-transfected cells (Fig. 7D).

Transfection with dominant negative receptor mutants that lack the tyrosine kinase domain [Ueno et al., 1992], [either cytoplasmic/nuclear FGFR1(TK-) or exclusively nuclear FGFR1(SP-/NLS)(TK-) (see Fig. 3A)] blocked dB-cAMP-induced differentiation of HNPC (Fig. 7E). In contrast, the extracellular FGFR antagonist IP6 had no effect on dB-cAMP-induced neurite outgrowth [Stachowiak et al., 2003]. These studies demonstrated that nuclear FGFR1 accumulation and the activation of INFS are essential for cAMP-induced neuronal differentiation [Stachowiak et al., 2003] and suggest that the tyrosine kinase domain of nuclear FGFR1 generates signals that are sufficient for both neurite outgrowth and gene transactivation associated with neuronal differentiation (see also next section). Stimulation of cellular differentiation by nuclear FGFR1 is a

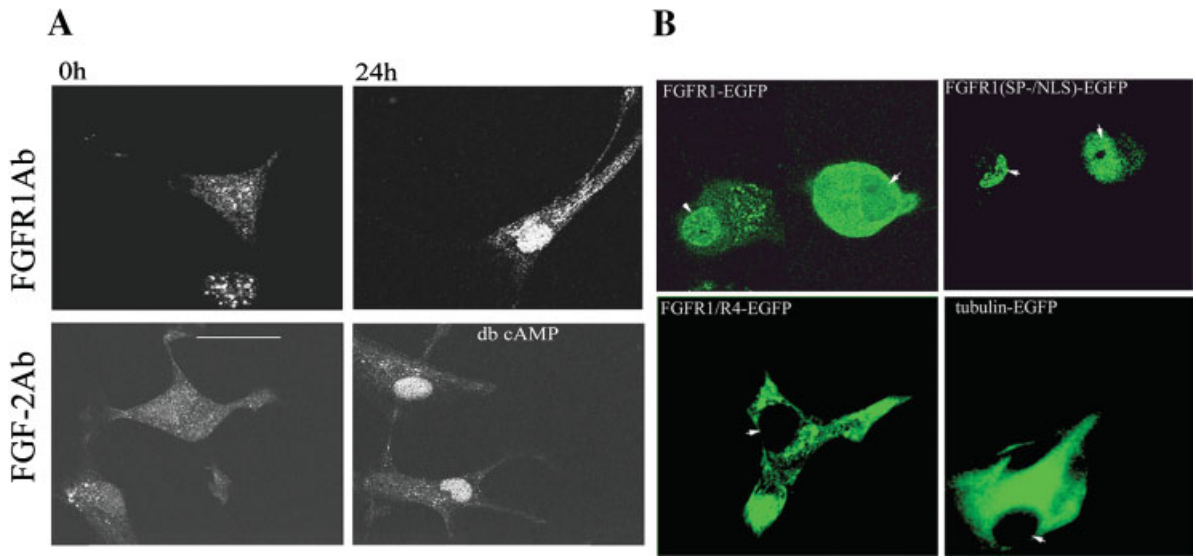

Fig. 7. Nuclear accumulation of fibroblast growth factor-2 (FGF-2) and FGR1 accompanies differentiation of human neuronal progenitor cells (HNPC). **A**: FGFR1 and FGF-2 accumulate in the nuclei of HNPC treated with 0.1 mM dB-cAMP. Images represent confocal sections approximately through the middle of the nucleus. The nuclear accumulation of FGF-2 and FGFR1 was observed by 6 h after the addition of dB-cAMP and preceded the onset of neurite outgrowth [not shown; Stachowiak et al., 2003]. **B**: Distinct subcellular localizations for FGFR1-EGFP (nuclear/cytoplasmic), FGFR1(SP-/NLS)-EGFP (exclusively nuclear), and FGFR1/R4-EGFP or tubulin-EGFP (non-nuclear) fusion proteins in transiently transfected HNPC [Stachowiak et al., 2003]. **C**: Effects of cytoplasmic/nuclear FGFR1, exclusively nuclear FGFR1(SP-/NLS), or membrane-associated FGFR1/R4 on neurite outgrowth. FGFR1 or its mutants were co-transfected with an EGFP-expressing plasmid to reveal the morphology of the transfected cells, and images were obtained 4 days later. Bar graphs, the length of the single longest process in individual fluorescent cells was measured. **D**: Transfected nuclear FGFR1 stimulates the expression of neuron specific enolase (NSE) and light neurofilament protein (Nf-L). HNPC were transfected with the marker plasmid pEGFP along with pcDNA3.1, FGFR1, or FGFR1(SP-/NLS). Forty-eight hours later, cells were immunostained with anti-α-internexin, anti-NF-l, or anti-NSE mAb and with rabbit-anti-mouse IgG-CY3. Fluorescent images of EGFP (depicting transfected cells) and of immunostaining (CY3, red) were acquired by fluorescence microscopy [from Stachowiak et al., 2003]. α-internexin is expressed constitutively by HNPC confirming their neuronal lineage [Stachowiak et al., 2003]. **E**: Dominant negative FGFR1 blocks dB-cAMP induced neurite outgrowth. Cells were transfected with the pEGFP marker plasmid and with cytoplasmic/nuclear FGFR1(TK-) or non-membranous, exclusively nuclear FGFR1(SP-/NLS)(TK-). Selected fluorescent images from Stachowiak et al. [2003] are shown. Bar graphs, the length of a single longest process in individual fluorescent cells was measured. Two-way ANOVA: ($P < 0.0005$) for dB-cAMP, transfected genes, and interaction between genes and dB-cAMP. Both cytoplasmic/nuclear FGFR1(TK-) and non-membrane, exclusively nuclear FGFR1(SP-/NLS)(TK-) blocked dB-cAMP-induced neurite outgrowth.

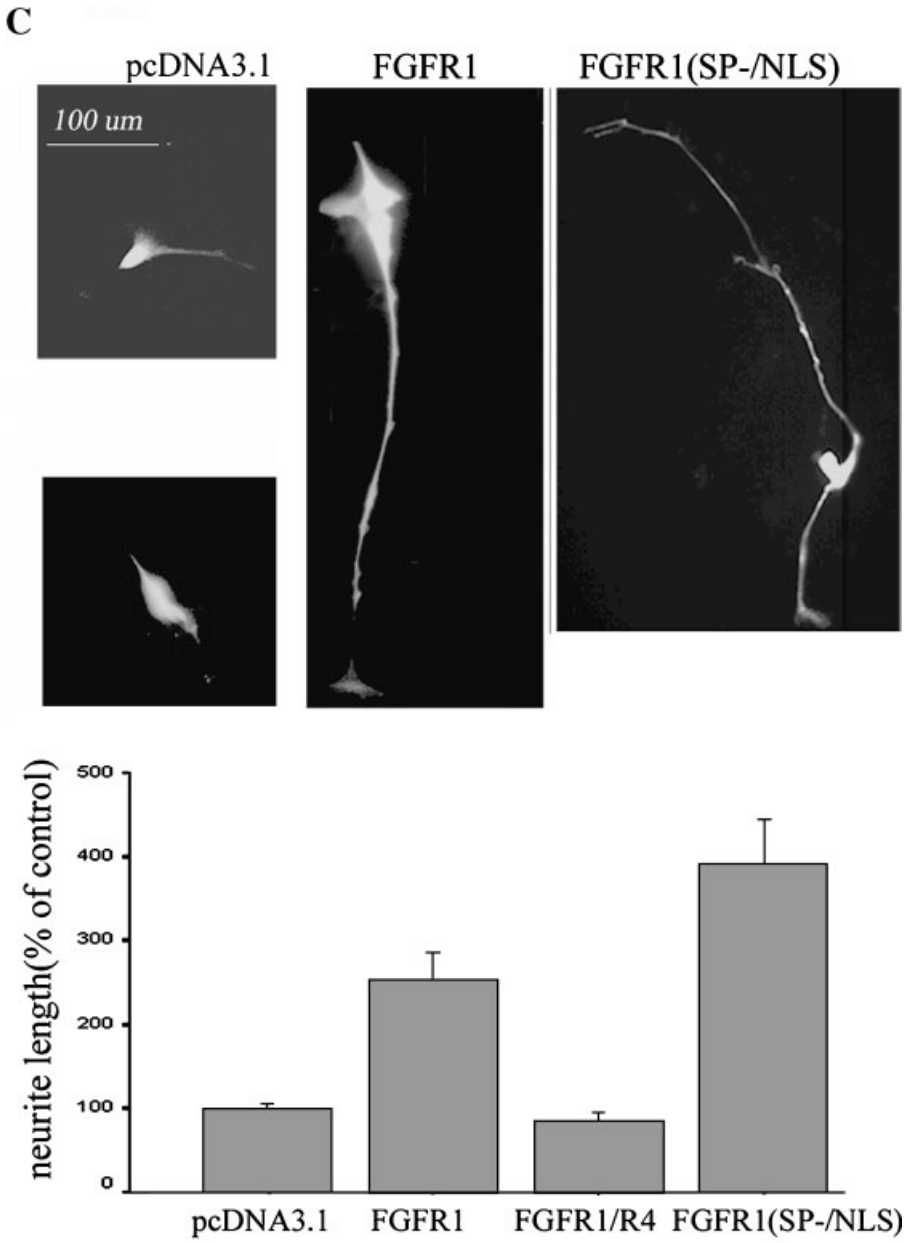

Fig. 7. (*Continued*)

common phenomenon occurring in different types of cells. For example, FGFR1(SP-/NLS) induced neuronal-like differentiation in TE671 cells, PC12 cells, SHSY5Y neuroblastoma cells [Stachowiak et al., 2003], and umbilical cord blood-derived neural stem cells [Stachowiak et al., 2003].

Bone morphogenetic proteins (BMPs) provide one of the physiological signals that control neurogenesis. BMP-7 induces differentiation of HNPC and dendritic growth in explanted sympathetic neurons from neonatal rats. BMP-7 signals through SMAD proteins that act as transcriptional factors, which associate with and are augmented by the transcriptional coactivators CBP/p300. Stimulation of BMP-7 receptors leads to an accumulation of SMADs and BMP-7-induced dendritic outgrowth is reduced by a dominant negative SMAD (Higgins). However, BMP-7 also induces the accumulation of FGFR1 and FGF-2 in the cell nuclei [Horbinski et al., 2002; Stachowiak et al., 2003]

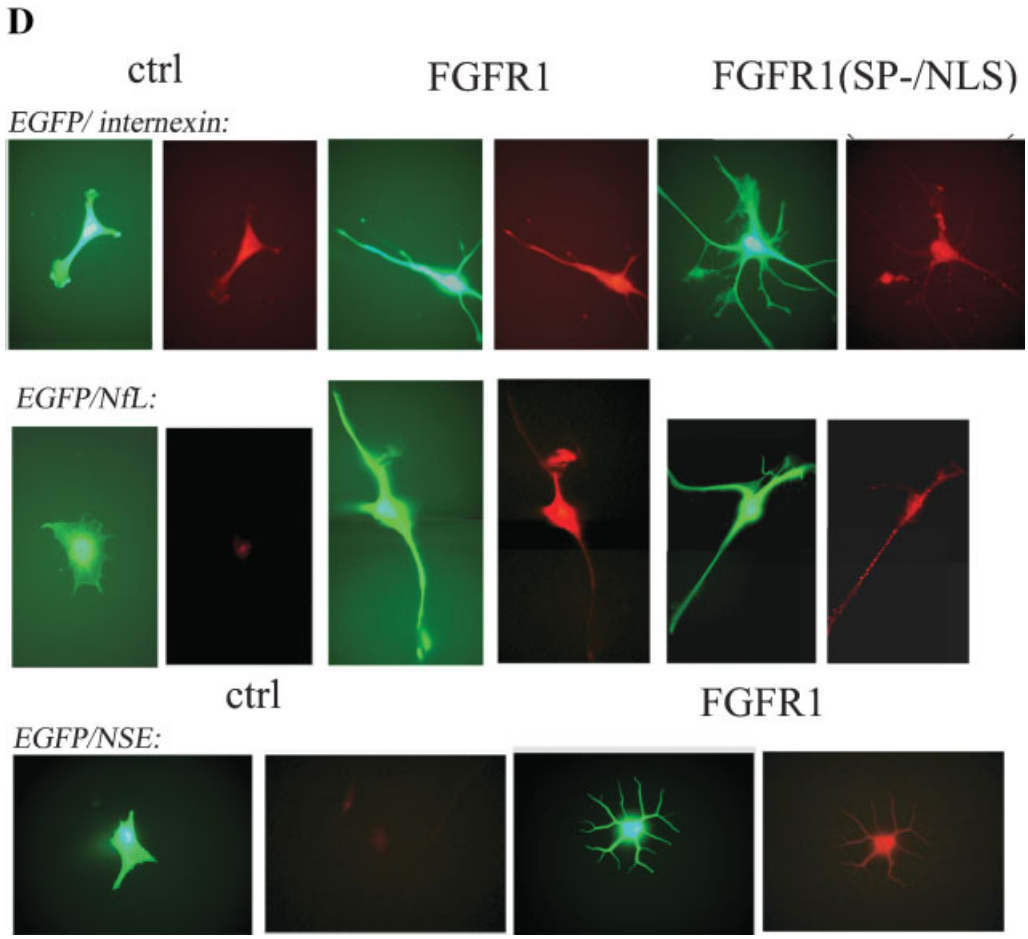

Fig. 7. (*Continued*)

and BMP-7-induced dendritic outgrowth is also inhibited by transfection with FGFR1(TK-) by ~50%. In contrast, targeted inhibition of extracellular FGFs by overexpression of a secreted mutant receptor lacking the transmembrane domain, FGFR1(TM-), failed to affect BMP-7-induced dendritic outgrowth, as did treatment with the extracellular FGFR antagonist, IP6. These results suggest that INFS and SMAD signaling are required for BMP-7 to stimulate dendritic development [Horbinski et al., 2002].

In summary, the nuclear accumulation of FGFR1 and its resulting effects on cell growth and differentiation were observed in many different types of cells and were induced by a variety of treatments, including changes in cell contact, addition of growth factors (i.e., BMP-7, EGF, AII, PDGF), stimulation of acetylcholine receptors, cell depolarization, and activation of different intracellular signaling pathways. Thus, we named it integrative nuclear fgfr1 signaling (INFS) [Peng et al., 2001, 2002; Stachowiak et al., 2003].

NUCLEAR FGFR1 AS A TRANSCRIPTIONAL REGULATOR

The nuclear targeting of FGFR1 suggested that FGFR1 might transduce diverse extracellular and intracellular signals directly to the genome. As the first step towards elucidating the mechanism of nuclear FGFR1 action, we analyzed the localization of FGFR1 in different sub-nuclear compartments. FGFR1 is associated with the nucleoplasm and nuclear matrix-nuclear lamina fraction (Fig. 8A). Immunoelectron microscopic analysis of in situ-fractionated adherent cells showed ~0.2 μm clusters of FGFR1 within the interior of the nuclear matrix suggesting that the receptor's intranuclear functions are associated with specific regions of the nuclear matrix [Stachowiak et al., 1996a]. The nuclear matrix consists

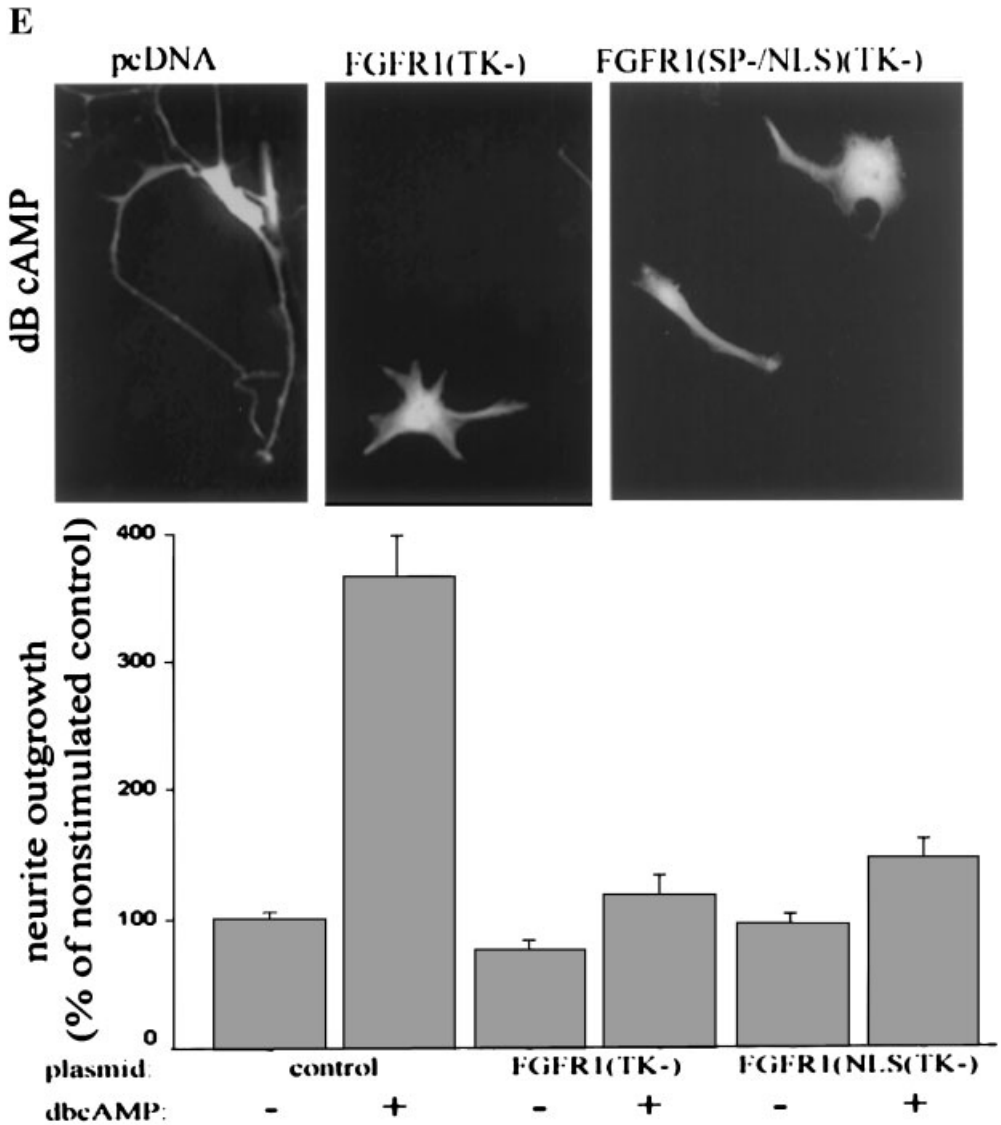

Fig. 7. (*Continued*)

of a web of internal fibers connected to the nuclear lamina [Berezney, 1980]. DNA replication [Berezney and Coffey, 1977], transcription [Jackson and Cook, 1985], and RNA processing [Zeitlin et al., 1987] are architecturally organized on the matrix. Topoisomerase, transcription factors [Eisenman et al., 1985; Chatterjee and Flint, 1986], and proteins that regulate the cell cycle [Chatterjee and Flint, 1986; Greenfield et al., 1991; Mancini et al., 1994] associate with the nuclear matrix. Therefore, our findings raise the intriguing possibility that FGFR1 may exert its influence over a number of cellular processes by acting within the environment of the nuclear matrix.

Confocal microscopy also showed that the nuclear accumulation of FGFR1-IR in response to cell stimulation had a speckled distribution [Peng et al., 2002; Stachowiak et al., 2003]. The exclusion of nuclear FGFR1 from BrdU-labeled sites in proliferating HPNC (Fig. 7A) and the transient (in G1-phase, Fig. 6B) nuclear accumulation of FGFR1 in astrocytes argue against a direct role for FGFR1 in DNA replication. Therefore, we hypothesized that nuclear FGFR1 could play a role in the activation of genes that prepare cells for DNA replication (see the next section).

Another type of nuclear speckle-like domain corresponds to sites of RNA Pol II-mediated

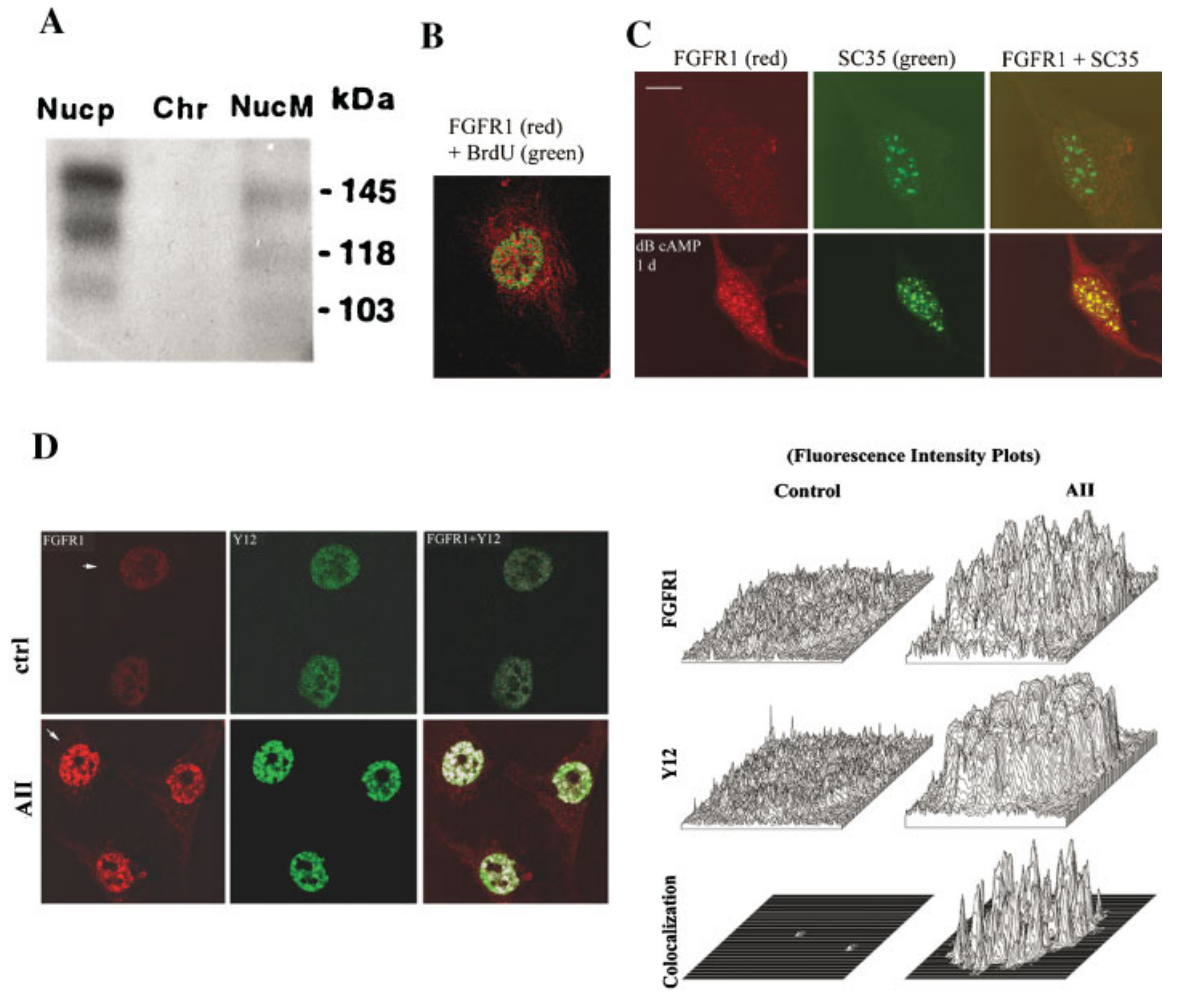

Fig. 8. Fibroblast growth factor receptor-1 (FGFR1) associates with the nuclear matrix and with RNA synthesis/processing domains but not with DNA replication sites. **A**: Western blot analysis of FGFR1 in nucleoplasm (Nucp), chromatin (Chr), and nuclear matrix (NucM) of human astrocytes [from Stachowiak et al., 1996b]. **B**: HNPC show an absence of FGFR1 colocalization with DNA replicons in proliferating cells. To mark DNA replication sites, the proliferating, non-differentiated HNPC were incubated with 10 μM BrdU for 12 h and then stained with anti-BrdU mAb-goat anti mouse IgG-Alexa 488 (green) and with C-term FGFR1 Ab-goat anti rabbit IgG-CY3 (red). Merged image shows no colocalization of FGFR1 with BrdU. **C**: HNPC, in differentiating cells FGFR1 is associated with splicing factor-enriched extranucleolar domains. HNPC were incubated with or without 100 μM dB-cAMP and stained with FGFR1 C-term Ab (red) and SC35 mAb (green). In cells treated with dB-cAMP, the intensity of SC35 and FGFR1 staining increased relative to non-treated cells and formed speckle-like domains. In the merged images, the yellow color indicates colocalized FGFR1 and SC35 pixels. **D**: In BAMC, angiotensin II (AII)-induced nuclear FGFR1 associates with nuclear Y12 rich domains. Left, in cells treated with AII, the intensity of Y12 as well as FGFR1 staining increased and formed speckle-like domains. The FGFR1 positive speckles overlap with the Y12 speckles. The merging of Y12 (green) and FGFR1 (red) staining; (white) colocalized FGFR1 with Y12 pixels. Single optical sections approximately through the middle of the BAMC nuclei are shown. Right, the emergence and colocalization of FGFR1 and Y12 speckle domains in AII-treated cells is illustrated by fluorescence intensity plots. FGFR1-IR pixels (85–98%) overlap with Y12-IR pixels indicating that nearly all FGFR1 is associated with the Y12-containing speckles. In contrast, in control cells, less than 10% of FGFR1 and Y12 pixels were colocalized and the pixels did not assemble into speckle-like domains [from Peng et al., 2002]. **E**: Colocalization of FGFR1 with sites of transcription (Ts), RNA Pol II (Pol II), and DNA replication (Rs). Nascent RNA Ts labeled by BrUTP incorporation or staining for hyperphosphorylated RNA Polymerase II show a typical punctate staining pattern consisting of several hundred foci in a typical mid plane nucleus (**top panel**). Merging of these images with those for FGFR1 shows limited colocalization between sites of transcription and FGFR1. **F**: To quantitate the extent of colocalization between FGFR1 with sites of transcription (Ts), RNA Pol II (Pol II), and DNA replication (Rs) we determined the area in pixels that overlap between the two segments, 37% of the area occupied by the large, granular FGFR1 sites showed colocalization with sites of transcription. In contrast, no colocalization of FGFR1 with BrdU labeled Rs is observed. Panels E and F were derived from Somanathan et al., 2003.

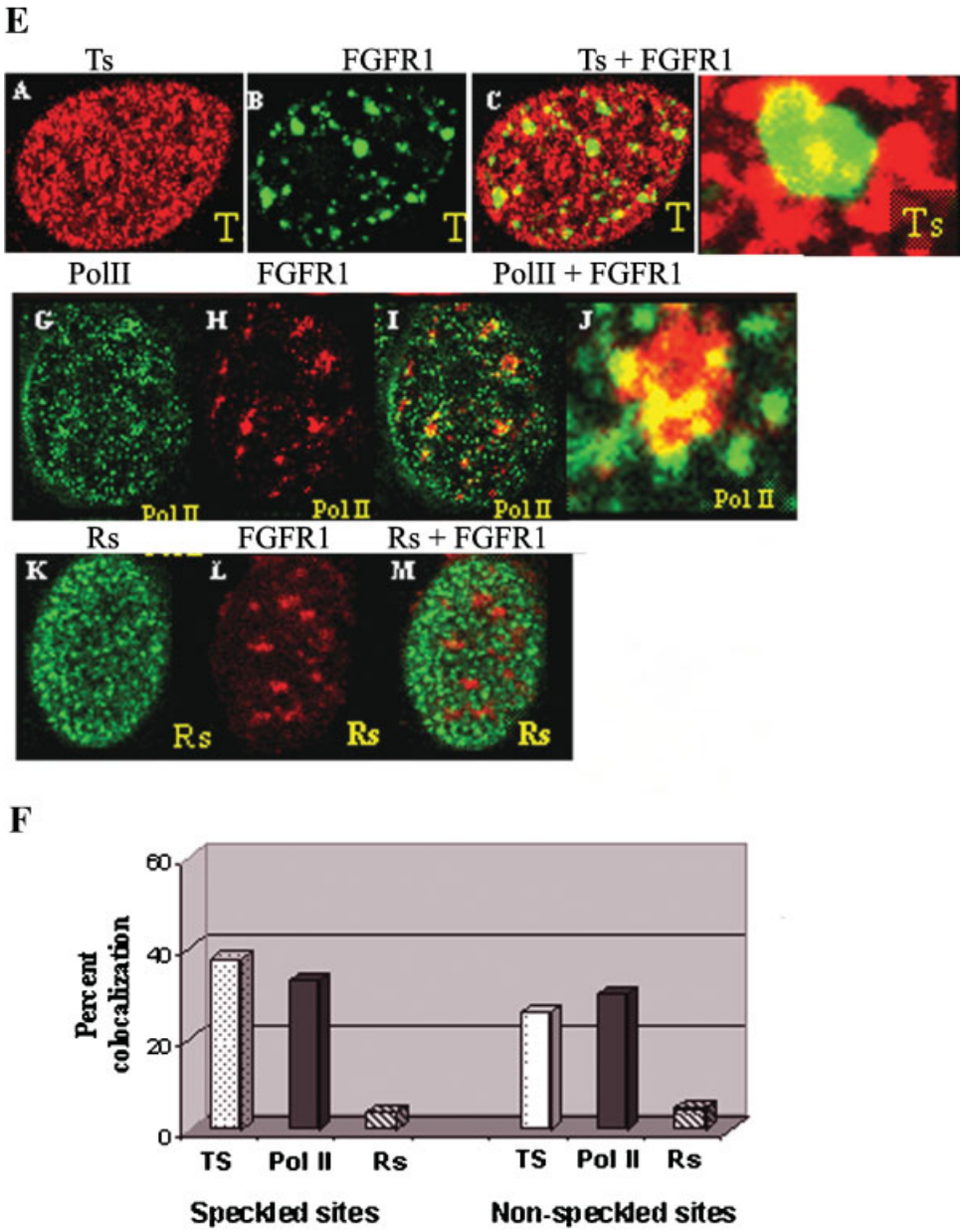

Fig. 8. (*Continued*)

transcription and co-transcriptional, pre-mRNA processing which are located on the periphery and in the interior of the speckles, respectively [Blencowe et al., 1994; Smith et al., 1999; Wei et al., 1999]. These domains can be labeled either with a monoclonal antibody raised against the spliceosome assembly factor, SC-35 [Blencowe et al., 1994] or with McAbY12, which recognizes snRNPs involved in RNA processing [Lerner et al., 1981]. These speckles are presumed to be equivalent to the extra-nucleolar nuclear matrix-attached granules observed in the EM [Fu and Maniatis, 1990; Wei et al., 1999]. The SC35 McAb detected speckles of variable intensity throughout the nucleus, with the exception of the nucleolus (Fig. 8B) in proliferating HNPC. Little or no colocalization between SC-35 speckles and FGFR1 grains was observed. In contrast, colocalization of SC35 and FGFR1 was observed in several larger aggregates of dB-cAMP-treated differentiating cells (Fig. 8C). Furthermore,

analysis of consecutive, confocal sections showed several examples of FGFR1 sites within the interior of the SC35 speckles [Stachowiak et al., 2003]. The FGFR1 nuclear speckles also overlapped three-dimensionally with McAbY12-stained nuclear domains. In non-stimulated BAMC, McAbY12-IR and FGFR1-IR were distributed in variable amounts throughout the nucleus showing less than 10% colocalization and were not assembled into speckle-like domains [Fig. 8D; Peng et al., 2002]. In contrast, after 1 h of AII treatment, several large speckles, which stained intensely with McAbY12 and FGFR1, appeared in the nuclei. Merging the McAbY12 and FGFR1 images confirmed that under these conditions, nearly all (89–85%) of the FGFR1 is associated with the McAbY12-containing speckles [Fig. 8D; Peng et al., 2002].

Histone H3 phosphorylation is restricted to a small fraction of H3 histone that is dynamically and highly acetylated and has been implicated in establishing the transcriptional competence of genes [Spencer and Davie, 1999]. Transfection of FGFR1 stimulated H3 phosphorylation and the transfected receptor colocalized with the sites of anti-phospho H3 Ab staining in TE671 cells, which express very low levels of endogenous FGFR1 [Stachowiak et al., 2003].

The specific localization of FGFR1 within nearly all of the SC35/McAbY12/phospho-H3-rich nuclear domains suggested that FGFR1 could be directly involved in regulating the expression of a large number of genes. To determine if FGFR1 associates specifically with sites of RNA transcription, serum-stimulated, proliferating human TE671 cells were permeabilized and labeled with BrUTP. Sites of BrUTP incorporation, representing nascent transcripts, and the localization of FGFR1 were compared. Confocal microscopy showed a limited co-localization of sites of transcription (Ts) and FGFR1. Sites of transcription were detected within and on the periphery of the FGFR1 nuclear speckles (Fig. 8E, Somanathan et al., 2003). In some instances, smaller FGFR1 speckles were either surrounded by sites of transcription or in close vicinity to one. Thirty-seven percent of the area occupied by the large, granular FGFR1 speckles showed co-localization with sites of transcription. In contrast, less than 5% of the FGFR1 speckles colocalized with DNA replication sites (Fig. 8F, Somanathan et al., 2003).

In summary, the colocalization of FGFR1 with sites of RNA synthesis and processing throughout the nucleus suggests that the receptor could act as a general transcriptional regulator that controls the activities of genes located at different sites throughout the genome.

INFS REGULATES THE ACTIVITIES OF STRUCTURALLY UNRELATED GENES LOCATED ON DIFFERENT CHROMOSOMES

The *FGF-2* gene, located on human chromosome 4, was the first gene shown to be regulated by nuclear FGFR1 [Peng et al., 2001]. Increases in FGF-2 protein synthesis and upregulation of FGF-2 mRNA were accompanied by the nuclear accumulation of endogenous FGFR1 and FGF-2 [Stachowiak et al., 1994b, 1996a,b; Moffett et al., 1998; Peng et al., 2001, 2002].

We have demonstrated that FGFR1 mediates the activation of the *FGF-2* gene promoter by acting downstream from a variety of extracellular factors and cAMP- and PKC-dependent signaling pathways [Peng et al., 2001]. The upregulation of endogenous *FGF-2* gene activity was prevented by FGFR1(TK-) [Fig. 9B; Peng et al., 2001]. In contrast, the extracellular FGFR antagonists, IP6, or suramin had no effect on the stimulation of the *FGF-2* gene by PMA and forskolin (Fig. 9C) or AII (Fig. 9B), indicating an intracellular site of action for the effect of FGFR1 on FGF-2 transcription. Furthermore, FGF-2 promoter activity and expression of the endogenous *FGF-2* gene were activated by transfection with wild type FGFR1 or FGFR1(SP-/NLS) (Fig. 9D). These effects were prevented by co-transfection with the dominant negative FGFR1(TK-) construct, but not by treatment with IP6 or suramin [Peng et al., 2001]. This demonstrated that an increase in the nuclear FGFR1 content is sufficient to activate the *FGF-2* gene.

Consistent with the implied participation of nuclear FGFR1 in the activation of the *FGF-2* gene by extracellular factors and cAMP and PKC signaling pathways, deletion of the upstream (−555/512 bp) FGF-2 promoter region which mediates the stimulation of the gene by AII, cAMP and PMA as well as regulatory sequences proximal to the transcription start site, reduced the transactivation of the FGF-2 promoter by transfected nuclear FGFR1 [Peng et al., 2001]. Furthermore, FGF-2 promoter activation by nuclear FGFR1 correlated with an

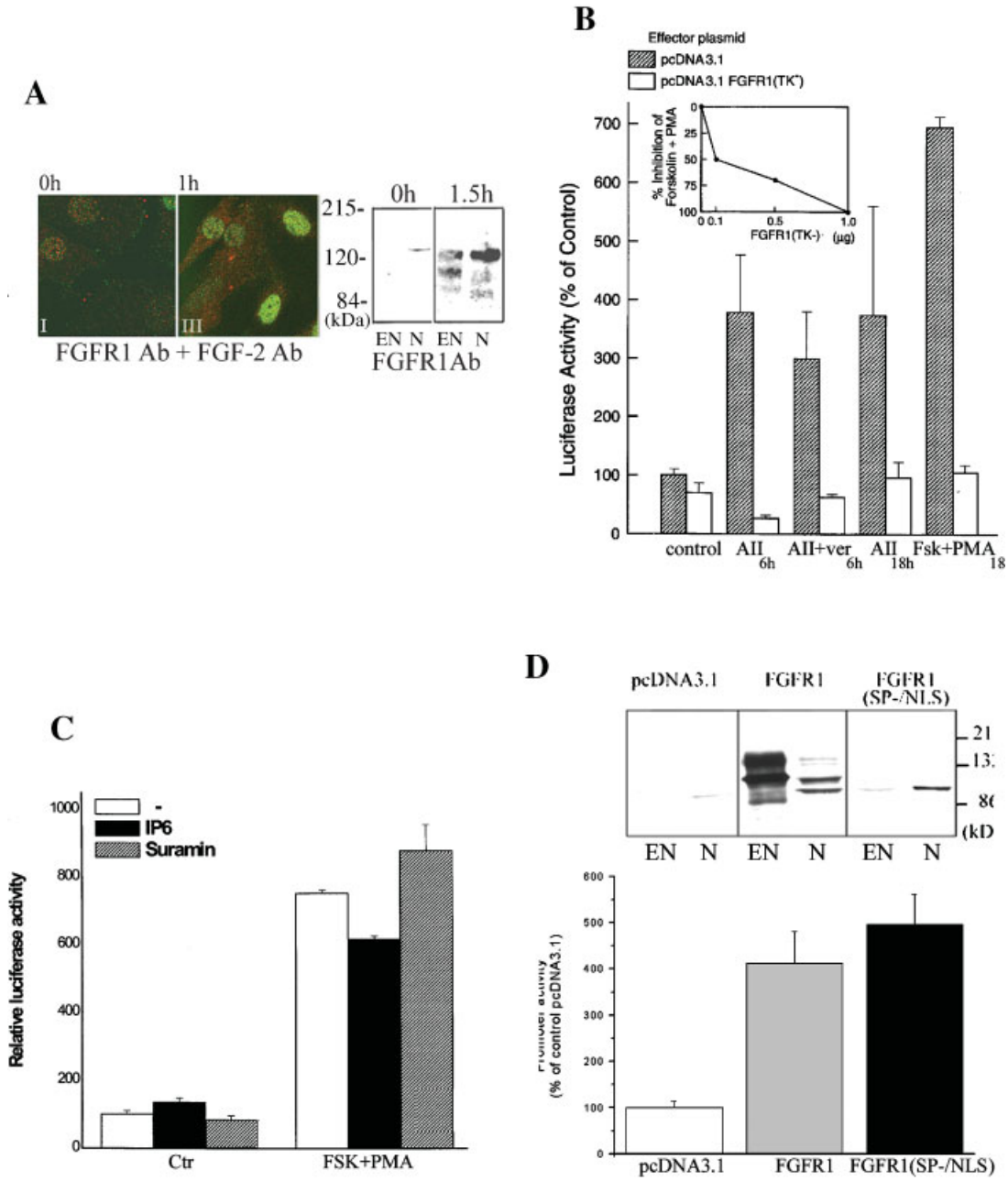

Fig. 9. Fibroblast growth factor receptor-1 (FGFR1) signaling is essential and sufficient for the activation of the *FGF-2* gene promoter [from Peng et al., 2002]. **A**: In serum-starved BAMC, nuclear AII stimulates the rapid nuclear accumulation of both FGFR1 (red) and FGF2 (green) in the same cells. The rapid nuclear localization of FGFR1 was confirmed by Western blot analysis of the nuclear (N) and extranuclear (EN) fractions with FGFR1 McAb6 [from Peng et al., 2002]. **B**: FGFR1(TK-) blocks the activation of the *FGF-2* gene promoter. BAMC were co-transfected with 1 μg of (−650/+314) FGF-2 promoter-Luc (FGF-2Luc) plasmid and either pcDNA3.1-FGFR1(TK-) or control pcDNA3.1 (1 μg each or with increasing concentrations, see insert). Two days later, transfected cells were incubated with 1 μM sar[1] AII, 5 μM veratridine, AII + veratridine, 0.1 μM PMA + 5 μM forskolin or in control, drug-free bbmedium for 24 h [from Peng et al., 2001]. **C**: Antagonists of cell surface FGFR, inositolhexakisphosphate (IP6), or suramin (50 μM) do not block activation of the *FGF-2* gene promoter by forskolin and PMA. BAMC were transfected with FGF-2Luc and IP6 (400 μM) or suramin (50 μM) were added 1 h before forskolin and PMA treatment. **D**: Transactivation of the *FGF-2* gene promoter by nuclear FGFR1. Inset, TE671 cells that express low levels of endogenous FGFR1 were transfected with a plasmid expressing a wild type FGFR1, a FGFR1 receptor mutant lacking the signal peptide but equipped with a nuclear localization signal [FGFR1(SP-/NLS)], or control pcDNA3.1 (1 μg each). Nuclear (N) and extranuclear (EN) fractions were analyzed by Western blotting with FGFR1 McAb6. Bar graph, FGF-2Luc (1 μg) was co-transfected with plasmids (1 μg) expressing FGFR1, FGFR1(SP-/NLS), or pcDNA3.1.

increase in in vitro protein binding to the −555/512 bp regulatory element. The results indicated that nuclear FGFR1 transactivates the *FGF-2* gene promoter by interacting with (directly or indirectly) the −555/512 bp element-binding protein.

Another gene shown to be regulated by nuclear FGFR1 encodes tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis. The *TH* gene is located on human chromosome 11 and is expressed specifically in catecholamine producing cells. Its

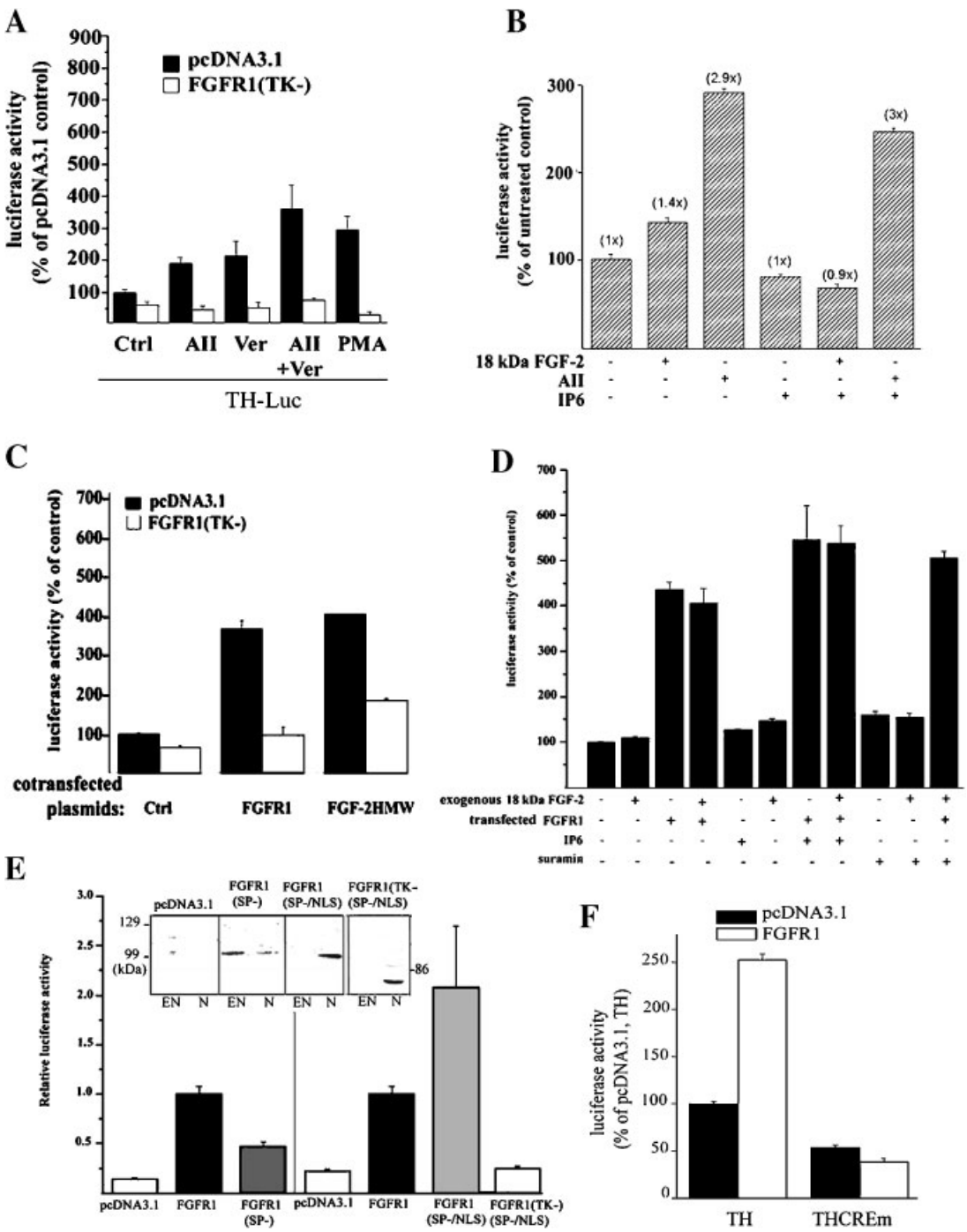

Fig. 10.

activity in both peripheral and central catecholaminergic cells is subjected to regulation by diverse homeostatic stimuli allowing lasting changes in catecholamine output. De novo expression of the *TH* gene protein observed in human neuronal stem/progenitor cells coincides with the nuclear accumulation of FGFR1 [Stachowiak et al., 2003]. Similarly, the upregulation of *TH* gene activity in BAMC by AII, veratridine, forskolin, or PMA coincided with the rapid nuclear accumulation of FGF-2 and FGFR1. The regulation of TH promoter-reporter constructs (TH-Luc) by a variety of stimuli parallels that of the endogenous *TH* gene, TH mRNA, and TH protein [Lewis et al., 1987; Stachowiak et al., 1990a,b, 1994a; Kilbourne et al., 1992; Goc and Stachowiak, 1994]. A dominant negative FGFR1(TK-) completely prevented the elevation in *TH* gene activity (Fig. 10A) as well as endogenous TH expression seen in BAMC in response to treatment with AII, veratridine, or PMA demonstrating that FGFR1 is essential for the transcriptional activation of the *TH* gene by various stimuli [Peng et al., 2002]. In contrast, the extracellular FGFR antagonist, IP6, did not prevent the activation of the *TH* gene promoter by those stimuli (while reducing stimulation by exogenous FGF-2) (Fig. 10B) suggesting that the promoter activation was mediated by intracellular FGFR1 [Peng et al., 2002].

Co-transfection of TE671 cells with the TH promoter-reporter construct and a plasmid that expresses nuclear HMWFGF-2 but not with one that expresses cytoplasmic FGF-1 induced the nuclear accumulation of FGFR1 and upregulated several-fold *TH* gene promoter activity (Fig. 10C). The TH promoter was also transactivated by co-transfection with wild type FGFR1 or FGFR1(SP-/NLS) but not by their inactive, TK-derivatives (Fig. 10E). In cells transfected with FGFR1, no additional stimulation of the TH promoter by exogenous FGF-2 was observed (Fig. 10D). Furthermore, the increase in TH-Luc expression in cells transfected with FGFR1 or nuclear HMWFGF-2 was not reduced by IP6 or suramin [Peng et al., 2002]. In addition to blocking the interaction between cell surface FGFR and extracelllular ligands, suramin also prevents ligand-induced receptor internalization [Moscatelli, 1988; Dai and Peng, 1995; Estival et al., 1996]. Thus, the absence of an effect of suramin on TH-Luc transactivation by FGFR1 or HMWFGF-2 indicates that the nuclear FGFR1 that mediates this transactivation is not derived from the cell surface through ligand-induced receptor internalization. These results are also consistent with the failure of biotinylated receptor to translocate from the cell surface to the nucleus [Peng et al., 2002] and with the model in which nuclear receptor is derived from the ER membranes, released into the cytosol and then transported by importin-β into the nucleus via the nuclear pores. Taken together, these data demonstrated that FGFR1 can transactivate the TH promoter by acting specifically in the cell nucleus.

Regulation of the TH promoter depends largely on a CREB-binding cAMP-responsive element (CRE), which plays the primary role in *TH* gene regulation by AII, veratridine, PMA, or cAMP. Mutation of the CRE prevented TH-Luc transactivation by FGFR1 (Fig. 10F) and by HMWFGF-2 (not shown) thus demonstrating that nuclear FGFR1 is engaged in the CRE-mediated regulation of *TH* gene activity [Peng et al., 2002].

Another gene that is regulated in a CRE-dependent manner and which is activated by nuclear FGFR1 is neurofilament-l (NF-l), which

Fig. 10. Activation of the TH promoter by AII, depolarization, and PKC is mediated by fibroblast growth factor receptor-1 (FGFR1) in an intracrine manner [from Peng et al., 2002]. **A**: Co-transfected FGFR1(TK-) blocks activation of a tyrosine hydroxylase promoter-Luc (TH-Luc) construct by AII, veratradine, and PMA. **B**: FGFR1-dependent activation of the TH promoter by AII occurs through an intracrine pathway. The extracellular FGFR antagonist IP6 blocks activation of TH-Luc by exogenous FGF-2 but not by AII. **C**: Transactivation of the *FGF-2* gene promoter by transfected, nuclear HMW-FGF-2 or by transfected FGFR1 is inhibited by FGFR1(TK-). TE671 cells were co-transfected with a plasmid expressing wild type FGFR1, HMW-FGF-2 or control vector and either FGFR1(TK-) or pcDNA3.1. **D**: Promoter activation by transfected FGFR1 does not involve cell surface FGFR1. Exogenous 18 kDa FGF-2 fails to activate TH-Luc in TE671 cells overexpressing FGFR1. Activation of the TH promoter by transfection with FGFR1 is not affected by blocking the activation of cell surface FGFR1 with suramin or IP6. **E**: Transactivation of the *TH* gene promoter by wild type FGFR1, non-membranous FGFR1(SP-), and by nuclear FGFR1(SP-/NLS). Inset shows the presence of transfected receptors in the nuclear (N) and extranuclear (EN) fractions from TE671 cells (Western blot with McAb6 FGFR1). Bar graph, TH-Luc (1 μg) was co-transfected with plasmids (1 μg) expressing FGFR1, FGFR1(SP-), or FGFR1(SP-/NLS), or with control pcDNA3.1. The luciferase activity was determined 48 h later and is expressed relative to the activity in cells transfected with FGFR1. **F**: Transactivation of the TH promoter (TH-Luc) by transfected FGFR1 is inhibited by mutation of theTH promoter CRE.

is located on human chromosome 8. Transfection of differentiating HNPC with FGFR1(SP-/NLS) increased the expression of both the endogenous *NF-l* gene and a CRE-containing *NF-l* gene promoter-β-galactosidase reporter construct [Stachowiak et al., 2003]. FGFR1(SP-/NLS) also transactivated a minimal TATA box promoter containing three tandem CRE repeats linked to the luciferase reporter demonstrating that it may serve as a general CRE transactivator [Stachowiak et al., 2003]. The transactivation of the CRE by nuclear FGFR1 required the presence of its tyrosine kinase domain.

Other genes shown to be regulated by nuclear FGFR1 are c-Jun and cyclin D1 [Reilly and Maher, 2001] located on chromosomes 1 and 11, respectively. The promoters of both genes contain a CRE, however the cis-elements that mediates their regulation by nuclear FGFR1 have not yet been determined. The activation of the *c-Jun* and *cyclin D1* genes provides a mechanism through which nuclear FGFR1 could stimulate entry into the cell cycle as observed in glial cells [Joy et al., 1997; Stachowiak et al., 1997].

INFS AS A REGULATOR OF CREB AND CBP

CRE binding protein (CREB) activates the expression of many nerve-specific genes including *NF-l*, *TH*, *chromogranin*, *pEJ*, and a number of immediate early genes that encode transcription factors (i.e., c-Jun). This causes the transactivation of other cis-acting elements and the spreading of the activation to gene promoters that lack a CRE [Impey and Goodman, 2001]. Phosphorylation of CREB by different protein kinases constitutes the final common step in pathways that mediate neurite outgrowth, neuronal differentiation, the control of neurotransmitter synthesis, and neurotransmission [Ginty et al., 1994; Tang et al., 1996; Murphy and Segal, 1997; Pugazhenthi et al., 1999; Schmid et al., 1999; White et al., 2000; Impey and Goodman, 2001; West et al., 2002, reviews]. CREB phosphorylation also controls growth and many other basic biological processes in non-neuronal cells in the same manner [Giles et al., 1998].

Nuclear FGFR1 was found to increase CREB activity twofold, which was accompanied by an upregulation of p44 phospho-CREB [Peng et al., 2002]. Maximal CREB transactivation of CRE requires the association of CREB with the limiting co-factor, CREB binding protein (CBP) [Impey and Goodman, 2001]. Phosphorylated CREB binds to the multifunctional transcriptional coactivator (CBP) that bridges DNA-bound CREB to the RNA polymerase II complex and modifies promoter-associated core histones through intrinsic CBP histone acetyltransferase (HAT) activity and by bringing CBP-associated

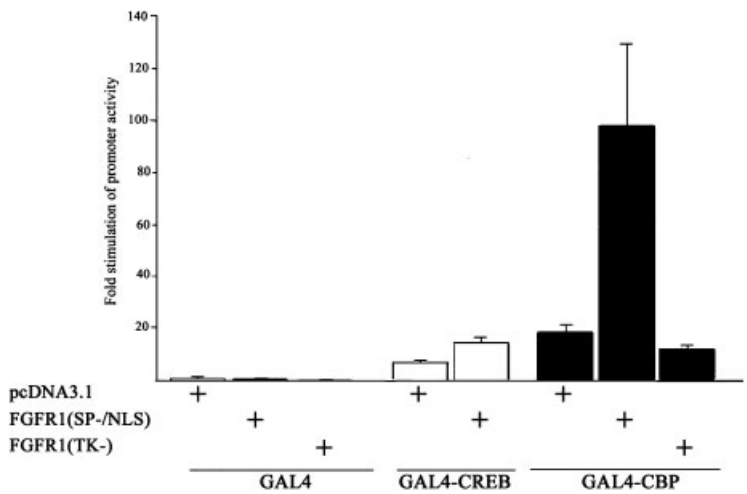

Fig. 11. Transactivation of GAL-Luc (pFR-Luc with GAL4-binding promoter) by GAL(1-147)-CREB or GAL(1-147)-CBP is augmented by FGFR1(SP-/NLS) but not by FGFR1(TK-) lacking the kinase domain. GAL(1-147)-CREB increased pFR-Luc expression sevenfold and together with FGFR1(SP-/NLS) increased transcription 15-fold when compared to pcDNA3.1 (twofold stimulation by FGFR1(SP-/NLS), $P < 0.05$). GAL(1-147)-CBP increased pFR-Luc activity 18-fold and together with FGFR1(SP-/NLS) >90-fold. FGFR1(SP-/NLS) had no significant effect on the expression of pFR-Luc in the presence of non-fused GAL4 [from Stachowiak et al., 2003].

chromatin-modifying proteins to DNA [Bannister and Kouzarides, 1996; Ogryzko et al., 1996; Giles et al., 1998; Shaywitz and Greenberg, 1999; Goodman and Smolik, 2000].

To determine if nuclear FGFR1 can also activate CBP, cells were co-transfected with pFR-Luc and the chimeric GAL4-CBP plasmid. Gal4-CBP stimulated pFR-Luc activity approximately 18-fold, and co-transfected FGFR1 (SP-/NLS) increased pFR-Luc stimulation to over 90-fold (Fig. 11). Thus, nuclear FGFR1 can stimulate transcription both by activating CREB and, even more effectively, by activating CBP. Transcriptional stimulation requires the TK domain of the receptor, since FGFR1(TK-) did not stimulate pFR-Luc transcription (Fig. 11) [Stachowiak et al., 2003].

Thus, one mechanism of gene activation by nuclear FGFR1 is through CBP. The FGFR1-responsive elements identified thus far include the −555/−512 bp element and the TRE in the FGF-2 promoter and the CRE in the TH and NF-1 promoters. All of these elements share a common ability to associate with CBP/p300 transcriptional co-activators and to be transactivated by CBP [Goodman and Smolik, 2000; Impey and Goodman, 2001; our unpublished observation]. In the *TH* gene promoter, transactivation of the CRE by nuclear HMWFGF-2 and FGFR1(SP-/NLS) was accompanied by the formation of multiple protein-DNA complexes with the *TH* gene promoter region containing the CRE and the TATA box element as observed in EMSA assays (Fig. 12) [Peng et al., 2002]. These complexes contained CREB and CBP/p300 as well as other, as yet to be identified, protein(s). Their formation was prevented by co-transfection with FGFR1(TK-) [Peng et al., 2002] and by direct incubation with an antibody to FGFR1 Ab (Fig. 12) indicating that nuclear FGFR1 may stimulate CBP-mediated transcription by enabling the formation of CREB-CBP-containing transcriptional complexes [Peng et al., 2002]. Our most recent studies indicate that nuclear FGFR1 may interact directly with these proteins [Fang et al., 2002].

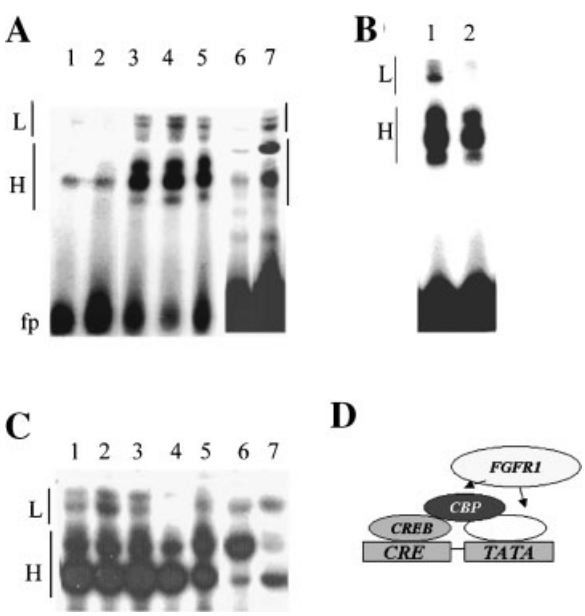

Fig. 12. The CRE-TATA box-associated protein complexes induced by nuclear fibroblast growth factor receptor-1 (FGFR1) or HMWFGF-2 contain CREB and CBP and their formation is affected by co-transfected FGFR1(TK-) or by incubation with anti-FGFR McAb6 [from Peng et al., 2002]. **A**: EMSA with the (−600/0 bp) TH promoter-CRE DNA probe and nuclear extracts (3 μg protein each) from TE671 transfected with HMWFGF-2, FGFR1 or control plasmids. Nuclear extracts were incubated for 30 min at room temperature with the ^{32}P-labeled TH promoter-CRE probe and analyzed by EMSA. **Lanes 1**, **2**: pBK; **Lanes 3**, **4**: HMWFGF; **Lane 5**: FGFR1; **Lane 6**: pcDNA3.1; **Lane 7**: FGFR1(SP-/NLS). Lanes 1–5 and 6, 7 represent separate experiments. ''L'' and ''H'' indicate low and high mobility retarded bands; fp, free probe. H and L complexes were competed out by an unlabeled CRE oligonucleotide (not shown). **B**: Nuclear extracts (3 μg each) from TE671 transfected with HMWFGF-2 were incubated with the ^{32}P-labeled TH promoter-CRE probe for 30 min at room temperature. Subsequently, the reactions were treated with antibodies (1 μg) for an additional 8 h at 4°C and then analyzed by EMSA. **Lane 1**, control monoclonal Ab; **lane 2**, FGFR1 McAb6. **C**: Nuclear extracts (3 μg each) from TE671 cells transfected with FGFR1 were incubated with the ^{32}P-labeled TH promoter-CRE probe for 30 min at room temperature. Subsequently, the reactions were treated with antibodies (1 μg) for an additional 8 h at 4°C and then analyzed by EMSA. **Lane 1**, no antibody; **lane 2**, control monoclonal Ab; **lane 3**, control polyclonal Ab; **lane 4**, FGFR1 McAb6; **lane 5**, C-term FGFR1 polyclonal Ab; **lane 6**, neutralizing CREB McAb; **lane 7**, CBP/p300 McAb. Neutralization of FGFR1 by McAb6 reduced the formation of the slowest CREB-CBP/p300-containing ''L'' complex as well as the ''L'' complex, which lacks CREB-CBP/p300. However, McAb6 had little or no effect on the smaller protein-DNA complexes found in the upper and lower ''H'' bands, which represent CREB and CBP/p300 protein-DNA complexes, respectively. This suggests that FGFR1 function is essential for the formation of large, multi-protein CRE complexes that include CREB and its cofactors CBP/p300, as well as other protein(s), but not for the smaller CREB- or CBP/p300-containing complexes. Formation of these large complexes was also prevented by transfection of FGFR1(TK-) [not shown; Peng et al., 2002]. **D**: The EMSA experiments indicate that nuclear FGFR1 may either associate directly with the CREB-CBP-containing complexes that bind to the proximal *TH* gene promoter region or that nuclear FGFR1 is essential for their formation [Peng et al., 2002].

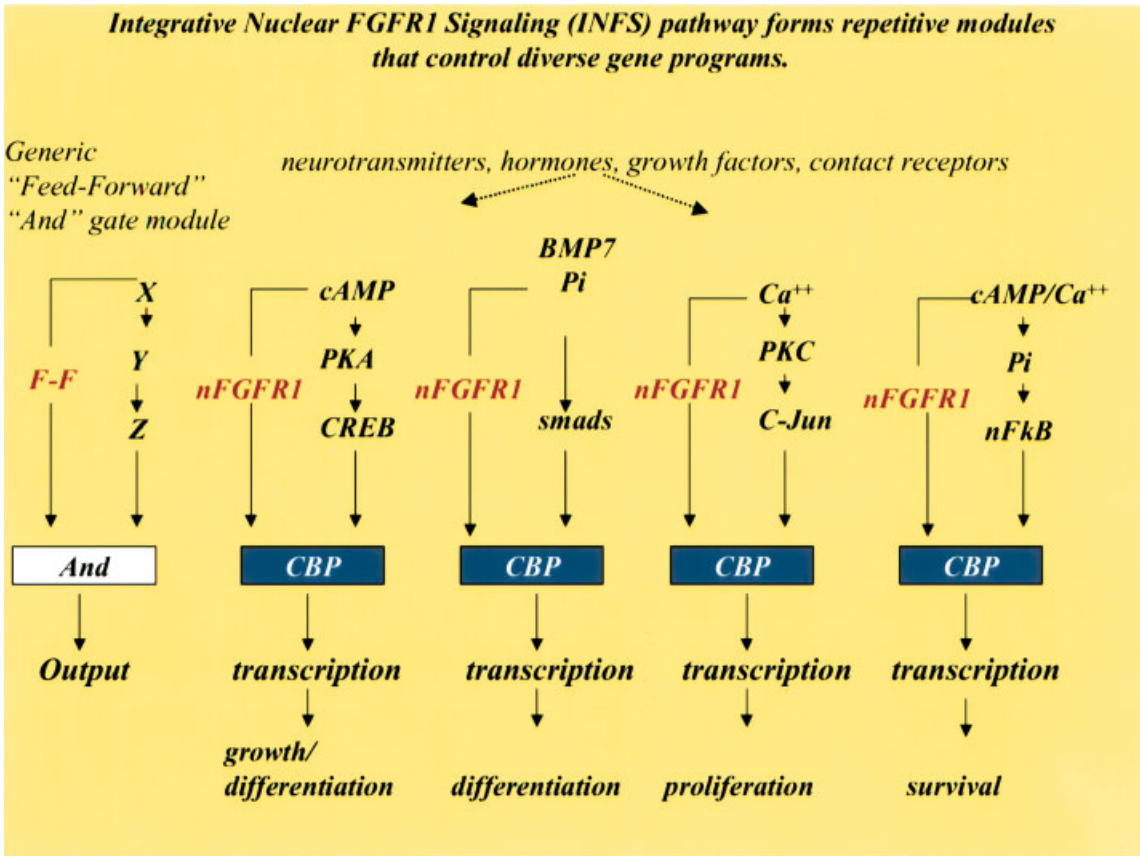

Fig. 13. We propose a universal ''feed-forward-and-gate'' signaling module in which classical signaling cascades initiated by specific membrane receptors transmit signals to sequence specific transcription factors (ssTFs), while the integrative nuclear FGFR1 signaling (INFS) elicited by these same stimuli feeds the signal forward to the common CBP co-activator. Activation of CBP by INFS along with the activation of ssTFs by classical signaling cascades could bring about the coordinated responses of structurally different genes.

CBP, and its close homologue p300, are transcriptional co-activators that integrate complex signal transduction events at the transcriptional level (110). CBP/p300 operate at the end points of cAMP and many other signaling pathways that transduce their activation to CREB, AP1, SMAD, NFkB, and a number of other sequence specific transcription factors (ssTFs). All these ssTFs bind CBP or p300, thereby enabling the formation of the transcription preinitiation complex (PIC) and essential chromatin modifications. These processes, consequently, allow transcriptional activation to take place. Many of the different cell surface receptors and signaling pathways that utilize CBP as their final transducer increase the nuclear accumulation of FGFR1. By acting as a general CBP activator, nuclear FGFR1 could link these specific signaling events to CBP and thereby permit transcriptional activation to take place. Thus, nuclear FGFR1 appears to constitute a universal gene transducer without which many surface receptors and their signaling pathways fail to activate CBP function. Consistent with this broad function, nuclear FGFR1 controls a diverse array of cellular processes including proliferation, growth, and differentiation, similar to CBP/p300 [Shaywitz and Greenberg, 1999; Goodman and Smolik, 2000; Impey and Goodman, 2001]. The mechanisms through which nuclear FGFR1 stimulates CBP function are currently under investigation.

To explain the control of multi-gene programs by FGFR1, we propose that nuclear FGFR1, together with specific signaling cascades, forms repetitive "Feed-Forward-And-Gate" signaling modules (Fig. 13). In such modules, a specific signaling cascade transmits signals from a receptor to ssTF(s) while FGFR1, which accumulates in the cell nucleus in response to the

same stimuli, feeds the signal forward to a common CBP co-activator. In this context, the function of INFS is to enable and/or augment transcriptional activation by ssTFs and to spread this activation to different, CBP-regulated genes. Further studies are needed to determine if the INFS feed forward mechanism operates in conjunction with ssTFs that are not CRE-binding proteins.

REFERENCES

Abraham JA, Mergia A, Whang JL, Tumolo A, Friedman J, Hjerrild KA, Gospodarowicz D, Fiddes JC. 1986a. Nucleotide sequence of a bovine clone encoding the angiogenic protein basic fibroblast growth factor. Science 233:545–548.

Abraham JA, Whang JL, Tunolo A, Mergia A, Friedman J, Gospodarowicz D, Fiddes JC. 1986b. Human basic fibroblast growth factor: nucleotide sequence and genomic organization. EMBO J 5:2523–2528.

Adam SA, Marr RS, Gerace L. 1990. Nuclear protein import in permeabilized mammalian cells requires soluble cytoplasmic factors. J Cell Biol 111:807–816.

Ali S, Davis MG, Becker MW, Dorn GW. 1993. Thromboxane A2 stimulates vascular smooth muscle hypertrophy by up-regulating the synthesis and release of endogenous basic fibroblast growth factor. J Biol Chem 268:17397–17403.

Ali S, Becker MW, Davis MG, Dorn GW. 1994. Dissociation of vasoconstrictor-stimulated basic fibroblast growth factor expression from hypertrophic growth in cultured vascular smooth muscle cells. Relevant roles of protein kinase C. Circ Res 75:836–843.

Arese M, Chen Y, Florkiewicz RZ, Gualandris A, Shen B, Rifkin DB. 1999. Nuclear activities of basic fibroblast growth factor: Potentiation of low-serum growth mediated by natural or chimeric nuclear localization signals. Mol Biol Cell 10:1429–1444.

Bannister AJ, Kouzarides T. 1996. The CBP co-activator is a histone acetyltransferase. Nature 384:641–643.

Berezney R. 1980. Fractionation of the nuclear matrix. I. Partial separation into matrix protein fibrils and a residual ribonucleoprotein fraction. J Cell Biol 85:641–650.

Berezney R, Coffey DS. 1977. Nuclear matrix. Isolation and characterization of a framework structure from rat liver nuclei. J Cell Biol 73:616–637.

Bikfalvi A, Klein S, Pintucci G, Quarto N, Mignatti P, Rifkin DB. 1995. Differential modulation of cell phenotype by different molecular weight forms of basic fibroblast growth factor: Possible intracellular signaling by the high molecular weight forms. J Cell Biol 129:233–243.

Bilak MM, Hossain WA, Morest DK. 2003. Intracellular fibroblast growth factor produces effects different from those of extracellular application on development of avian cochleovestibular ganglion cells in vitro. J Neurosci Res 71:629–647.

Blencowe BJ, Nickerson JA, Issner R, Penman S, Sharp PA. 1994. Association of nuclear matrix antigens with exon-containing splicing complexes. J Cell Biol 127:593–607.

Bouche G, Gas N, Prats H, Baldin V, Tauber JP, Teissie J, Amalric F. 1987. Basic fibroblast growth factor enters the nucleolus and stimulates the transcription of ribosomal genes in ABAE cells undergoing G0—-G1 transition. Proc Natl Acad Sci USA 84:6770–6774.

Chatterjee PK, Flint SJ. 1986. Partition of E1A proteins between soluble and structural fractions of adenovirus-infected and -transformed cells. J Virol 60:1018–1026.

Clarke WE, Berry M, Smith C, Kent A, Logan A. 2001. Coordination of fibroblast growth factor receptor 1 (FGFR1) and fibroblast growth factor-2 (FGF-2) trafficking to nuclei of reactive astrocytes around cerebral lesions in adult rats. Mol Cell Neurosci 17:17–30.

Dai Z, Peng HB. 1995. Presynaptic differentiation induced in cultured neurons by local application of basic fibroblast growth factor. J Neurosci 15:5466–5475.

Delafontaine P, Lou H. 1993. Angiotensin II regulates insulin-like growth factor I gene expression in vascular smooth muscle cells. J Biol Chem 268:16866–16870.

Delrieu I. 2000. The high molecular weight isoforms of basic fibroblast growth factor (FGF-2): An insight into an intracrine mechanism. FEBS Lett 468:6–10.

Dono R, Zeller R. 1994. Cell-type-specific nuclear translocation of fibroblast growth factor-2 isoforms during chicken kidney and limb morphogenesis. Dev Biol 163: 316–330.

Eisenman RN, Tachibana CY, Abrams HD, Hann SR. 1985. V-myc- and c-myc-encoded proteins are associated with the nuclear matrix. Mol Cell Biol 5:114–126.

Estival A, Monzat V, Miquel K, Gaubert F, Hollande E, Korc M, Vaysse N, Clemente F. 1996. Differential regulation of fibroblast growth factor (FGF) receptor-1 mRNA and protein by two molecular forms of basic FGF. Modulation of FGFR-1 mRNA stability. J Biol Chem 271: 5663–5670.

Evans J, Sumners C, Moore J, Huentelman MJ, Deng J, Gelband CH, Shaw G. 2002. Characterization of mitotic neurons derived from adult rat hypothalamus and brain stem. J Neurophysiol 87:1076–1085.

Fang X, Dunham S, Roy I, Stachowiak MK. 2002. Mechanisms of regulation gene expression by nuclear FGFR1 receptor. 2002. Soc Neurosci Annual Meeting 253.5, Orlando, Fl.

Fischer TA, Ungureanu-Longrois D, Singh K, de Zengotita J, DeUgarte D, Alali A, Gadbut AP, Lee MA, Balligand JL, Kifor I, Smith TW, Kelly RA. 1997. Regulation of bFGF expression and ANG II secretion in cardiac myocytes and microvascular endothelial cells. Am J Physiol 272:H958–H968.

Florkiewicz RZ, Baird A, Gonzalez AM. 1991. Multiple forms of bFGF: Differential nuclear and cell surface localization. Growth Factors 4:265–275.

Fu X-D, Maniatis T. 1990. Factor required for mammalian spliceosome assembly is localized to discrete regions in the nucleus. Nature 343:437–444. J 9:919–925.

Giles RH, Peters DJ, Breuning MH. 1998. Conjunction dysfunction: CBP/p300 in human disease. Trends Genet 14:178–183.

Ginty DD, Bonni A, Greenberg ME. 1994. Nerve growth factor activates a Ras-dependent protein kinase that stimulates c-fos transcription via phosphorylation of CREB. Cell 77:713–725.

Goc A, Stachowiak MK. 1994. Bovine tyrosine hydroxylase gene-promoter regions involved in basal and angiotensin

II-stimulated expression in nontransformed adrenal medullary cells. J Neurochem 62:834–843.
Gonzalez AM, Berry M, Maher PA, Logan A, Baird A. 1995. A comprehensive analysis of the distribution of FGF-2 and FGFR1 in the rat brain. Brain Res 701:201–226.
Goodman RH, Smolik S. 2000. CBP/p300 in cell growth, transformation, and development. Genes Dev 14:1553–1577.
Greenfield I, Nickerson J, Penman S, Stanley M. 1991. Human papillomavirus 16 E7 protein is associated with the nuclear matrix. Proc Natl Acad Sci USA 88:11217–11221.
Guo-Hong L. 1998. Inhibitory effects of antisense basic fibroblast growth factor oligonucleotides on proliferation of cultured aortic smooth muscle cells induced by angiotensin II in SHR rats. Acta Pharmacol Sin 19(2): 132–135.
Hanneken A, Maher PA, Baird A. 1995. High affinity immunoreactive FGF receptors in the extracellular matrix of vascular endothelial cells—Implications for the modulation of FGF-2. J Cell Biol 128:1221–1228.
Horbinski C, Stachowiak EK, Chandrasekaran V, Miuzukoshi E, Higgins D, Stachowiak MK. 2002. Bone morphogenetic protein-7 stimulates initial dendritic growth in sympathetic neurons through an intracellular fibroblast growth factor signaling pathway. J Neurochem 80:54–63.
Hou JZ, Kan MK, McKeehan K, McBride G, Adams P, McKeehan WL. 1991. Fibroblast growth factor receptors from liver vary in three structural domains. Science 251:665–668.
Imamura T, Engleka K, Zhan X, Tokita Y, Forough R, Roeder D, Jackson A, Maier JA, Hla T, Maciag T. 1990. Recovery of mitogenic activity of a growth factor mutant with a nuclear translocation sequence. Science 249: 1567–1570.
Imamura T, Haruta T, Takata Y, Usui I, Iwata M, Ishihara H, Ishiki M, Ishibashi O, Ueno E, Sasaoka T, Kobayashi M. 1998. Involvement of heat shock protein 90 in the degradation of mutant insulin receptors by the proteasome. J Biol Chem 273:11183–11188.
Impey S, Goodman RH. 2001. CREB signaling—Timing is everything. Sci.STKE 2001:E1.
Itoh H, Mukoyama M, Pratt RE, Gibbons GH, Dzau VJ. 1993. Multiple autocrine growth factors modulate vascular smooth muscle cell growth response to angiotensin II. J Clin Invest 91:2268–2274.
Jackson DA, Cook PR. 1985. Transcription occurs at a nucleoskeleton. EMBO J 4:919–925.
Jans DA. 1994. Nuclear signaling pathways for polypeptide ligands and their membrane receptors? FASEB J 8:841–847.
Jaye M, Howk R, Burgess WH, Ricca GA, Chiu I-M, Ravera MW, O'Brien SJ, Modi WS, Maciag T, Drohan WN. 1986. Human endothelial cell growth factor: cloning, nucleotide sequence, and chromosome localization. Science 233: 543–545.
Johnson DE, Williams LT. 1993. Structural and functional diversity in the FGF receptor multigene family. Adv Cancer Res 60:1–41.
Joy A, Moffett J, Neary K, Mordechai E, Stachowiak EK, Coons S, Rankin-Shapiro J, Florkiewicz RZ, Stachowiak MK. 1997. Nuclear accumulation of FGF-2 is associated with proliferation of human astrocytes and glioma cells. Oncogene 14:171–183.
Kilbourne EJ, Nankova BB, Lewis EJ, McMahon A, Osaka H, Sabban DB, Sabban EL. 1992. Regulated expression of the tyrosine hydroxylase gene by membrane depolarization. Identification of the responsive element and possible second messengers. J Biol Chem 267:7563–7569.
Klimaschewski L, Meisinger C, Grothe C. 1999. Localization and regulation of basic fibroblast growth factor (FGF-2) and FGF receptor-1 in rat superior cervical ganglion after axotomy. J Neurobiol 38:499–506.
Lerner EA, Lerner MR, Janeway CA, Jr, Steitz JA. 1981. Monoclonal antibodies to nucleic acid-containing cellular constituents: Probes for molecular biology and autoimmune disease. Proc Natl Acad Sci USA 78:2737–2741.
Lewis EJ, Harrington CA, Chikaraishi DM. 1987. Transcriptional regulation of the tyrosine hydroxylase gene by glucocorticoid and cyclic AMP. Proc Natl Acad Sci USA 84:3550–3554.
Li GH, Yang GJ. 1998. Inhibitory effect of antisense basic fibroblast growth factor oligonucleotides on proliferation of cultured aortic smooth muscle cells induced by angiotensin II in SHR rats. Zhongguo Yao Li Xue Bao 19:132–135.
Maher PA. 1996. Nuclear translocation of fibroblast growth factor (FGF) receptors in response to FGF-2. J Cell Biol 134:529–536.
Mancini MA, Shan B, Nickerson JA, Penman S, Lee WH. 1994. The retinoblastoma gene product is a cell cycle-dependent, nuclear matrix-associated protein. Proc Natl Acad Sci USA 91:418–422.
Matlack KE, Mothes W, Rapoport TA. 1998. Protein translocation: Tunnel vision. Cell 92:381–390.
Matsuda S, Okumura N, Yoshimura H, Koyama Y, Sakanaka M. 1992a. Basic fibroblast growth factor-like immunoreactivity in Purkinje cells of the rat cerebellum. Neuroscience 50:99–106.
Matsuda S, Desaki J, Okumura N, Shiosaka S, Imaoka S, Sakanaka M. 1992b. Basic fibroblast growth factor-like immunoreactivity in the trigeminal proprioceptive and motor systems. Brain Res 577:92–100.
Moffett J, Kratz E, Florkiewicz R, Stachowiak MK. 1996. Promoter regions involved in density-dependent regulation of basic fibroblast growth factor gene expression in human astrocytic cells. Proc Natl Acad Sci USA 93:2470–2475.
Moffett J, Kratz E, Myers J, Stachowiak EK, Florkiewicz RZ, Stachowiak MK. 1998. Transcriptional regulation of fibroblast growth factor-2 expression in human astrocytes: Implications for cell plasticity. Mol Biol Cell 9: 2269–2285.
Morrison RS, Shi E, Kan M, Yamaguchi F, McKeehan W, Rudnicka-Nawrot M, Palczewski K. 1994. Inositolhexakisphosphate (InsP6): An antagonist of fibroblast growth factor receptor binding and activity. In Vitro Cell Dev Biol Anim 30A:783–789.
Moscatelli D. 1988. Metabolism of receptor-bound and matrix-bound basic fibroblast growth factor by bovine capillary endothelial cells. J Cell Biol 107:753–759.
Murphy DD, Segal M. 1997. Morphological plasticity of dendritic spines in central neurons is mediated by activation of cAMP response element binding protein. Proc Natl Acad Sci USA 94:1482–1487.

Myers JM, Martins GG, Ostrowski J, Stachowiak MK. 2003. Nuclear trafficking of FGFR1: a role for the transmembrane domain. J Cell Biochem 88:1273–1291.

Nakanishi Y, Kihara K, Mizuno K, Masamune Y, Yoshitake Y, Nishikawa K. 1992. Direct effect of basic fibroblast growth factor on gene transcription in a cell-free system. Proc Natl Acad Sci USA 89:5216–5220.

Ogryzko VV, Schiltz RL, Russanova V, Howard BH, Nakatani Y. 1996. The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell 87: 953–959.

Olsnes S, Klingenberg O, Wiedlocah A. 2003. Transport of exogenous growth factors and cytokines to the cytosol and to the nucleus. Physiol Rev 83:163–182.

Peng H, Moffett J, Myers J, Fang X, Stachowiak EK, Maher P, Kratz E, Hines J, Fluharty SJ, Mizukoshi E, Bloom DC, Stachowiak MK. 2001. Novel nuclear signaling pathway mediates activation of fibroblast growth factor-2 gene by type 1 and type 2 angiotensin II receptors. Mol Biol Cell 12:449–462.

Peng H, Myers J, Fang X, Stachowiak EK, Maher PA, Martins GG, Popescu G, Berezney R, Stachowiak MK. 2002. Integrative nuclear FGFR1 signaling (INFS) pathway mediates activation of the tyrosine hydroxylase gene by angiotensin II, depolarization and protein kinase C. J Neurochem 81:506–524.

Piper DR, Mujtaba T, Keyoung H, Roy NS, Goldman SA, Rao MS, Lucero MT. 2001. Identification and characterization of neuronal precursors and their progeny from human fetal tissue. J Neurosci Res 66:356–368.

Powell PP, Klagsbrun M. 1991. Three forms of rat basic fibroblast growth factor are made from a single mRNA and localize to the nucleus. J Cell Physiol 148:202–210.

Puchacz E, Stachowiak EK, Florkiewicz RZ, Lukas RJ, Stachowiak MK. 1993. Basic fibroblast growth factor (bFGF) regulates tyrosine hydroxylase and proenkephalin mRNA levels in adrenal chromaffin cells. Brain Res 610:39–52.

Pugazhenthi S, Boras T, O'Connor D, Meintzer MK, Heidenreich KA, Reusch JE. 1999. Insulin-like growth factor I-mediated activation of the transcription factor cAMP response element-binding protein in PC12 cells. Involvement of p38 mitogen-activated protein kinase-mediated pathway. J Biol Chem 274:2829–2837.

Reilly JF, Maher PA. 2001. Importin beta-mediated nuclear import of fibroblast growth factor receptor: Role in cell proliferation. J Cell Biol 152:1307–1312.

Renko M, Quarto N, Morimoto T, Rifkin DB. 1990. Nuclear and cytoplasmic localization of different basic fibroblast growth factor species. J Cell Physiol 144:108–114.

Romisch K. 1999. Surfing the Sec61 channel: Bidirectional protein translocation across the ER membrane. J Cell Sci 112(23):4185–4191.

Schechter JE. 1992. Is cellular disruption the mechanism of release of basic fibroblast growth factor from anterior pituitary gonadotropes? Tissue Cell 24:791–802.

Schmid RS, Graff RD, Schaller MD, Chen S, Schachner M, Hemperly JJ, Maness PF. 1999. NCAM stimulates the Ras-MAPK pathway and CREB phosphorylation in neuronal cells. J Neurobiol 38:542–558.

Schweigerer L, Neufeld G, Mergia A, Abraham JA, Fiddes JC, Gospodarowicz D. 1987. Basic fibroblast growth factor in human rhabdomyosarcoma cells: Implications for the proliferation and neovascularization of myoblast-derived tumors. Proc Natl Acad Sci USA 84:842–846.

Shaywitz AJ, Greenberg ME. 1999. CREB: A stimulus-induced transcription factor activated by a diverse array of extracellular signals. Annu Rev Biochem 68: 821–861.

Sherman L, Stocker KM, Morrison R, Ciment G. 1993. Basic fibroblast growth factor (bFGF) acts intracellularly to cause the transdifferentiation of avian neural crest-derived Schwann cell precursors into melanocytes. Development 118:1313–1326.

Shin J, Lee S, Strominger JL. 1993. Translocation of TCR alpha chains into the lumen of the endoplasmic reticulum and their degradation. Science 259:1901–1904.

Smith KP, Moen PT Jr, Wydner KL, Coleman JR, Lawrence JB. 1999. Processing of endogenous pre-mRNAs in association with SC-35 domains is gene specific. J Cell Biol 144:617–629.

Somanathan S, Stachowiak EK, Siegel AJ, Stachowiak MK, Berezney R. 2003. A nuclear matrix bound fibroblast growth factor receptor is associated with splicing factor rich and transcriptionaly active nuclear speckles. J Cell Biochem 90: this issue.

Spencer VA, Davie JR. 1999. Role of covalent modifications of histones in regulating gene expression. Gene 240: 1–12.

Stachowiak MK, Hong JS, Viveros OH. 1990a. Coordinate and differential regulation of phenylethanolamine N-methyltransferase, tyrosine hydroxylase and proenkephalin mRNAs by neural and hormonal mechanisms in cultured bovine adrenal medullary cells. Brain Res 510: 277–288.

Stachowiak MK, Jiang HK, Poisner AM, Tuominen RK, Hong JS. 1990b. Short and long term regulation of catecholamine biosynthetic enzymes by angiotensin in cultured adrenal medullary cells. Molecular mechanisms and nature of second messenger systems. J Biol Chem 265:4694–4702.

Stachowiak MK, Goc A, Hong JS, Poisner A, Jiang HK, Stachowiak EK. 1994a. Regulation of tyrosine hydroxylase gene expression in depolarized non-transformed bovine adrenal medullary cells: Second messenger systems and promoter mechanisms. Brain Res Mol Brain Res 22:309–319.

Stachowiak MK, Moffett J, Joy A, Puchacz E, Florkiewicz R, Stachowiak EK. 1994b. Regulation of bFGF gene expression and subcellular distribution of bFGF protein in adrenal medullary cells. J Cell Biol 127:203–223.

Stachowiak MK, Maher PA, Joy A, Mordechai E, Stachowiak EK. 1996a. Nuclear accumulation of fibroblast growth factor receptors is regulated by multiple signals in adrenal medullary cells. Mol Biol Cell 7:1299–1317.

Stachowiak MK, Maher PA, Joy A, Mordechai E, Stachowiak EK. 1996b. Nuclear localization of functional FGF receptor 1 in human astrocytes suggests a novel mechanism for growth factor action. Brain Res Mol Brain Res 38:161–165.

Stachowiak EK, Maher PA, Tucholski J, Mordechai E, Joy A, Moffett J, Coons S, Stachowiak MK. 1997. Nuclear accumulation of fibroblast growth factor receptors in human glial cells—Association with cell proliferation. Oncogene 14:2201–2211.

Stachowiak MK, Moffett J, Maher PA, Tucholski J, Stachowiak EK. 1998. Growth factor regulation of cell

growth and proliferation in the nervous system. A new intracrine nuclear mechanism. Mol Neurobiol 15:1–27.

Stachowiak EK, Fang X, Myers J, Dunham S, Stachowiak MK. 2003. cAMP-induced differentiation of human neuronal progenitor cells is mediated by nuclear FGF receptor-1 (FGFR1). J Neurochem 84:1296–1312.

Szebenyi G, Fallon JF. 1999. Fibroblast growth factors as multifunctional signaling factors. Int Rev Cytol 185: 45–106.

Tang K, Wu H, Mahata SK, Taupenot L, Rozansky DJ, Parmer RJ, O'Connor DT. 1996. Stimulus-transcription coupling in pheochromocytoma cells. Promoter region-specific activation of chromogranin a biosynthesis. J Biol Chem 271:28382–28390.

Ueno H, Gunn M, Dell K, Tseng A, Jr, Williams L. 1992. A truncated form of fibroblast growth factor receptor 1 inhibits signal transduction by multiple types of fibroblast growth factor receptor. J Biol Chem 267:1470–1476.

Vlodavsky I, Bar-Shavit R, Ishai-Michaeli R, Bashkin P, Fuks Z. 1991. Extracellular sequestration and release of fibroblast growth factor: A regulatory mechanism? Trends Biochem Sci 16(7):268–271.

Wei X, Somanathan S, Samarabandu J, Berezney R. 1999. Three-dimensional visualization of transcription sites and their association with splicing factor-rich nuclear speckles. J Cell Biol 146:543–558.

West AE, Griffith EC, Greenberg ME. 2002. Regulation of transcription factors by neuronal activity. Nat Rev Neurosci 3:921–931.

White DM, Walker S, Brenneman DE, Gozes I. 2000. CREB contributes to the increased neurite outgrowth of sensory neurons induced by vasoactive intestinal polypeptide and activity-dependent neurotrophic factor. Brain Res 868: 31–38.

Wiedlocha A, Falnes PO, Madshus IH, Sandvig K, Olsnes S. 1994. Dual mode of signal transduction by externally added acidic fibroblast growth factor. Cell 76: 1039–1051.

Wiertz EJ, Jones TR, Sun L, Bogyo M, Geuze HJ, Ploegh HL. 1996. The human cytomegalovirus US11 gene product dislocates MHC class I heavy chains from the endoplasmic reticulum to the cytosol. Cell 84:769–779.

Wilkie AO, Morriss-Kay GM, Jones EY, Heath JK. 1995. Functions of fibroblast growth factors and their receptors. Curr Biol 5:500–507.

Woodward WR, Nishi R, Meshul CK, Williams TE, Coulombe M, Eckenstein FP. 1992. Nuclear and cytoplasmic localization of basic fibroblast growth factor in astrocytes and CA2 hippocampal neurons. J Neurosci 12:142–152.

Yang M, Omura S, Bonifacino JS, Weissman AM. 1998. Novel aspects of degradation of T cell receptor subunits from the endoplasmic reticulum (ER) in T cells: Importance of oligosaccharide processing, ubiquitination, and proteasome-dependent removal from ER membranes. J Exp Med 187:835–846.

Zeitlin S, Parent A, Silverstein S, Efstratiadis A. 1987. Pre-mRNA splicing and the nuclear matrix. Mol Cell Biol 7:111–120.