Journal of Cellular Biochemistry 88:1214–1225 (2003)

Nuclear Localization of Basic Fibroblast Growth Factor is Mediated by Heparan Sulfate Proteoglycans Through Protein Kinase C Signaling

Edward Hsia,[1] Thomas P. Richardson,[1] and Matthew A. Nugent[1,2]*

[1]Department of Biochemistry, Boston University School of Medicine, Boston, Massachusetts 02118
[2]Department of Ophthalmology, Boston University School of Medicine, Boston, Massachusetts 02118

Abstract Understanding the process of wound healing will provide valuable insight for the development of new strategies to treat diseases associated with improper regeneration, such as blindness induced by corneal scarring. Heparan sulfate proteoglycans (HSPG) are not normally expressed in the corneal stroma, but their presence at sites of injury suggests their involvement in the wound healing response. Primary cultured corneal stromal fibroblasts constitutively express HSPG and represent an injured phenotype. Recently, nuclear localization of HSPG was shown to increase in corneal stromal fibroblasts plated on fibronectin (FN), an extracellular matrix protein whose appearance in the corneal stroma correlates with injury. One possible role for the nuclear localization of HSPG is to function as a shuttle for the nuclear transport of heparin-binding growth factors, such as basic fibroblast growth factor (FGF-2). Once in the nucleus, these growth factors might directly modulate cellular activities. To investigate this hypothesis, cells were treated with ^{125}I-labelled FGF-2 under various conditions and fractionated. Our results show that nuclear localization of FGF-2 was increased in cells plated on FN compared to those on collagen type I (CO). Interestingly, FGF-2-stimulated proliferation was increased in cells plated on FN compared to CO and this effect was absent in the presence of heparinase III. Furthermore, pre-treatment with heparinase III decreased nuclear FGF-2, and CHO cells defective in the ability to properly synthesize heparan sulfate chains showed reduced nuclear FGF-2 indicating that the heparan sulfate chains of HSPG are critical for this process. HSPG signaling, particularly through the cytoplasmic tails of syndecans, was investigated as a potential mechanism for the nuclear localization of FGF-2. Treatment with phorbol 12-myristate-13-acetate (PMA), under conditions that caused downregulation of protein kinase Cα (PKCα), decreased nuclear FGF-2. Using pharmacological inhibitors of specific PKC isozymes, we elucidated a potential mode of regulation whereby PKCα mediates the nuclear localization of FGF-2 and PKCδ inhibits it. Our studies suggest a novel mechanism in which FGF-2 translocates to the nucleus in response to injury. J. Cell. Biochem. 88: 1214–1225, 2003. © 2003 Wiley-Liss, Inc.

Key words: FGF-2; heparan sulfate; proteoglycans; fibronectin; corneal fibroblasts; syndecan

Wound healing following injury is a complex and incompletely understood process. Understanding this process would be invaluable in developing new strategies to treat diseases associated with improper regeneration, such as blindness induced by corneal scarring, [Whitcher et al., 2001], or uncontrolled healing, such as cancer [Lotti et al., 1998; Barcellos-Hoff, 2001]. Growth factors, such as basic fibroblast growth factor (FGF-2), are involved in processes associated with wound healing such as cell migration, proliferation, and differentiation [Nugent and Iozzo, 2000; Okada-Ban et al., 2000]. FGF-2 can modulate these cell activities in a variety of ways. The best-characterized mechanism of FGF-2 action is through binding to its tyrosine kinase receptors leading to the consequent activation of a number of intracellular signaling pathways [Szebenyi, 1999]. Efficient activation of FGF receptors depends on the coordinated binding of FGF-2 to cell surface heparan sulfate proteoglycans (HSPG) [Park et al., 2000]. The interaction of FGF-2

Grant sponsor: NIH; Grant number: EY14007; Grant sponsor: Research to Prevent Blindness; Grant sponsor: Massachussetts Lions Eye Research Fund, Inc.

*Correspondence to: Matthew A. Nugent, Department of Biochemistry, Room K420, Boston University School of Medicine, 715 Albany Street, Boston, MA 02118.
E-mail: nugent@biochem.bumc.bu.edu

Received 9 December 2002; Accepted 10 December 2002
DOI 10.1002/jcb.10470

© 2003 Wiley-Liss, Inc.

with the structurally complex heparan sulfate chains has been shown to enhance FGF-2 receptor binding and signal transduction [Kwan et al., 2001; Turnbull et al., 2001; Esko and Selleck, 2002]. In contrast to these cell surface events, there have also been reports of the nuclear localization of FGF-2 and direct interaction with nuclear machinery in the stimulation of cell growth [Amalric et al., 1994; Jans and Hassan, 1998; Sperinde and Nugent, 1998; Keresztes and Boonstra, 1999; Bailly et al., 2000]. For example, FGF-2 interacts with and activates casein kinase-2, a ubiquitous serine/threonine kinase involved in the control of cell proliferation at the nuclear level [Bailly et al., 2000]. The mechanism by which FGF-2 translocates to the nucleus remains unclear but is thought to involve HSPG.

While HSPG are known to modulate the activities of FGF-2 by stabilizing receptor binding [Tumova and Couchman, 2000; Pellegrini, 2001], a growing body of evidence suggests additional roles for HSPG in FGF-2 mitogenesis. Syndecans, a family of transmembrane cell surface HSPG, are particularly interesting because of their wide distribution and their ability to initiate intracellular signaling through highly conserved cytoplasmic tails [Volk et al., 1999; Rapraeger, 2000; Simons and Horowitz, 2001]. The variable region within the cytoplasmic domain of syndecan-4, in particular, has been shown to bind and activate PKCα [Oh et al., 1997b; Horowitz, 1998a]. This interaction requires oligomerization of syndecan-4 cytoplasmic tails and phosphatidylinositol 4,5-bisphosphate, which is upregulated by fibronectin [Oh et al., 1997a; Oh et al., 1998; Horowitz et al., 1999]. Furthermore, PKCδ may regulate PKCα activation by phosphorylating a specific serine within the cytoplasmic tail of syndecan-4, thereby inhibiting the oligomerization necessary for PKCα activation [Horowitz, 1998a,b; Horowitz et al., 2002]. HSPG-mediated PKC signaling may play an important role in modulating FGF-2 activity.

A possible mechanism for the modulation of FGF-2 activity by HSPG could be the nuclear localization of FGF-2 through an HSPG-dependent pathway. FGF-2 has already been shown to be internalized through an HSPG-mediated pathway, independent of FGF receptors [Quarto, 1994]. In addition, reports of heparan sulfate in the nucleus suggest intracellular roles for HSPG, such as the regulation of growth at the transcriptional level [Fedarko and Conrad, 1986; Ishihara et al., 1986; Fedarko et al., 1989; Rykova and Grigorieva, 1998; Cheng et al., 2001]. Recently, HSPG has been shown to localize to the nucleus in corneal stromal fibroblasts (CSF) under conditions associated with an injured state [Richardson et al., 2000]. The corneal stroma is an excellent model to study HSPG-mediated wound healing processes since HSPG are not constitutively expressed in the uninjured stroma yet have been detected at the wound edge upon disruption [Hassell et al., 1983; Funderburgh and Chandler, 1989; Brown et al., 1995]. Because of the ability for HSPG to interact with FGF-2 and the presence of both FGF-2 and HSPG in the nucleus, we hypothesize that one role for intracellular HSPG may be to direct FGF-2 to the nucleus, and that this process may be regulated by PKC signaling.

In this report, we describe increased nuclear localization of FGF-2 in cells plated on FN compared to CO and show that this effect is decreased in the presence of heparinase III. In addition, FGF-2 stimulated proliferation was also increased in cells plated on FN compared to CO and this effect was abrogated upon treatment with heparinase III. Moreover, FGF-2 nuclear localization was decreased in a mutant CHO cell line defective in the ability to properly synthesize heparan sulfate chains. Through the use of PKC modulators, we uncovered a potential mechanism whereby the HSPG-mediated nuclear localization of FGF-2 requires PKCα and is inhibited by PKCδ. These findings suggest a novel mechanism by which FGF-2 is processed in response to injury in order to promote cell growth.

METHODS

Reagents

Human recombinant FGF-2 was a generous gift from Dr. W. Michael Kavanaugh of Chiron Corp. (Emeryville, CA). Preparation of ^{125}I-FGF-2 with ^{125}I-Bolton-Hunter reagent (NEN Life Science Products, Boston, MA) was conducted as previously described [Nugent and Edelman, 1992]. Fetal bovine serum (FBS) and cell culture reagents were from Life Technologies (Gaithersburg, MD). Fibronectin from bovine plasma was from Sigma (St. Louis, MO). Collagen I (bovine dermis) was from Becton Dickinson Labware (Bedford, MA). ITS™ culture

supplement was from BD Biosciences (Bedford, MA). Secondary antibodies, sheep anti-mouse IgG-horseradish peroxidase, and donkey anti-rabbit IgG-horseradish peroxidase, were from Amersham (Piscataway, NJ). Heparinase III from *Flavobacterium heparinum* was a generous gift from Biomarin Pharmaceuticals (Montreal, Canada). Gö 6976, Bisindolylmaleimide I, and rottlerin, were from Calbiochem (San Diego, CA). Phorbol 12-myristate 13-acetate (PMA) was from Sigma. Nonidet P-40 [NP-40; (polyethylene glycol)-*p*-isocytyophenyl ether; octylphenoxypolyethoxyethanol], and other reagent grade chemicals were from Sigma.

Cell Culture

Primary stromal fibroblasts (CSF) were isolated from rabbit corneas. Corneas were removed from fresh, whole rabbit eyes (Pel-Freez, Rogers, AS), rinsed in Dulbecco's modified Eagle's Medium (DMEM), low glucose, 100 μg/ml penicillin, 100 U/ml streptomycin, 0.8% Nystatin, and digested in collagenase A (2 mg/ml) from *Clostridium histolyticum* (Boehringer-Mannheim, Indiananapolis, IN) for 1.5 h at 37°C. After gently teasing away the epithelial layer, the partially digested corneas were transferred into fresh collagenase A and incubated for an additional 3.5 h at 37°C. Following centrifugation at 1,000*g* for 10 min and removal of supernatant, cells were resuspended and plated at 50,000/cm^2 in DMEM, low glucose, 0.1 mM MEM non-essential amino acids, 100 μg/ml penicillin, 100 U/ml streptomycin, 1% Nystatin, 10% FBS. After 2–3 days, cells were maintained in DMEM containing 4% FBS. When cells reached confluence, they were used for experiments. For all experiments, cells were trypsinized and plated at 15,000/cm^2 (unless otherwise stated) in serum-free DMEM and allowed to adhere overnight. Bacteriological dishes were prepared by coating with individual matrix molecules (fibronectin and collagen I) at 20 μg/ml in PBS for 16 h at 4°C. Consistent coating of different sized dishes was achieved by maintaining a 250 μl:1 cm^2 ratio for each well substratum [Ingber, 1990; Asthagiri et al., 1999]. Prior to cell plating, dishes were washed with serum-free DMEM at 37°C. CHO cells (CHO-K1 and -ldlD obtained from Dr. Monty Krieger at MIT, Cambridge, MA) were maintained in F-12 (Ham's) media supplemented with 100 μg/ml penicillin, 100 U/ml streptomycin, 5% dialyzed FBS. For experiments, CHO cells were plated in serum-free F-12 (Ham's) media supplemented with 1% ITS™ with and without 20 μM galactose overnight at 37°C [Krieger et al., 1989].

Cellular Fractionation

To quantitate the amount of ^{125}I-FGF-2 translocated to the nucleus under various conditions, cells were disrupted and fractionated through a modification of a previously described procedure [Maher, 1996; Sperinde and Nugent, 1998]. This method has been demonstrated to isolate nuclei with limited contamination by plasma membrane and cytosolic components. Experiments were initiated by washing the cells once with binding buffer (DMEM, low glucose, 25 mM HEPES, 0.1% BSA) at 37°C. Cells were treated as described in the legends of each figure and incubated with ^{125}I-FGF-2 at 37°C. After incubation with ^{125}I-FGF-2, cells were washed three times with ice-cold binding buffer to remove non-cell associated ^{125}I-FGF-2. Prior to cellular fractionation, cell surface associated ^{125}I-FGF-2 was removed. HSPG-associated ^{125}I-FGF-2 was released by extraction with a high-salt buffer (20 mM HEPES, 2 M NaCl, pH 7.4; 0.5 ml/well for 5 sec at RT) followed by a wash with PBS. Cell surface receptor-associated ^{125}I-FGF-2 was released by extraction with a high salt/acid buffer (20 mM sodium acetate, 2 M NaCl, pH 4.0; 0.5 ml/well for 5 min at RT), followed by a wash with PBS. Cells were trypsinized using 0.5 ml of 0.01% trypsin, 0.53 mM EDTA (Life Technologies, Inc.). Trypsinization was stopped by the addition of 0.5 ml/well of 1 mg/ml soybean trypsin inhibitor (Sigma) in binding buffer. Cell suspensions were placed in a Model 235C microcentrifuge (Fisher Scientific, NJ) and centrifuged for 30 sec at 10,000*g*. Cell pellets were resuspended by briefly vortexing in 200 μl of homogenization buffer (10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF) and incubated on ice for 15 min. After this incubation, 12.5 μl of a 10% NP-40 solution (in H_2O) was added, and the samples were vortexed vigorously for 10 sec. This was followed by another centrifugation, 30 sec at 10,000*g*. The supernatant was collected as the cytoplasmic fraction. The crude nuclear pellet was washed again by repeating the procedure outlined above. Radioactivity in these fractions was quantitated by counting in a Cobra II® Series Gamma Counter (Packard Corp.,

Meriden, CT). Counts were normalized based on cell number. To control for the carry-over of ^{125}I-FGF-2 from the cytoplasmic fraction into the nuclear pellet, we measured the amount of ^{125}I-FGF-2 added to crude cell extracts that became associated with the nuclear fraction. Three concentrations of ^{125}I-FGF-2 were used and the maximal amount of carry-over was determined to be 5%. Thus, 5% of the counts obtained from the cytoplasmic fraction were subtracted from the counts obtained from the nuclear pellet in all subsequent fractionation data.

Cell Proliferation Analysis

Cells were plated onto bacteriological dishes coated with 20 μg/ml FN or CO and allowed to attach and spread overnight at 37°C in serum-free DMEM. Treatment with heparinase III (0.4 U/ml) and/or FGF-2 (55 pM) were initiated 16 h after plating and additional doses of heparinase III and FGF-2 were added each day throughout the experiment. Cell number was determined at each time point using a particle count and size analyzer (Coulter Corp, Miami, FL). In control experiments, heparinase III activity loss was measured over time at 37°C and its half-life was determined to be 24 h [Buczek-Thomas et al., 2002].

Immunoblot Analysis

To identify changes in PKCα expression in the presence of PMA, cells were treated with PMA at 37°C for various times. Cultures were homogenized by scraping in 300 μl/well of extraction buffer (20 mM HEPES, pH 7.0, 1% Triton X-100, 10% glycerol, 2 mM PMSF), and nuclei and insoluble debris were removed by centrifugation at 12,000*g* for 10 min [Fannon, 1996]. Cell extracts were subjected to SDS–PAGE (8.5%) under reducing conditions, and the proteins were transferred to Immobilon-P (Millipore Corp, Bedford, MA). Membranes were blocked in Tris-buffered saline (TBS), 1% Tween-20, 5% nonfat milk for 1 h at RT (or overnight at 4°C) and probed for 1 h at 37°C with the following primary antibodies diluted in TBS, pH 7.6, 4% BSA, 0.03% NaN_3: (1) a rabbit polyclonal antibody to phosphorylated PKCα (Upstate Biotechnology, Inc., Lake Placid, NY) at 2 μg/ml; (2) a mouse monoclonal antibody for total PKCα (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) at 1 μg/ml; and (3) a mouse monoclonal antibody for β-actin (Sigma) at 1.35 μg/ml. Incubation with the primary antibody was followed by 1 h incubation at 37°C with horseradish peroxidase-linked secondary antibodies. Bands were visualized with ECL chemiluminescence (Amersham) on Hyperfilm ECL (Amersham). Blots could be stripped by submerging the membrane in stripping buffer (62.5 mM tris-HCl, pH 6.8, 2% SDS, 100 mM 2-mercaptoethanol) at 50°C for 30 min with gentle agitation, and re-probed. Autoradiographs were analyzed using Scion Image for Windows (Scion Corp., Frederick, MD) to determine relative band intensities.

RESULTS

Nuclear Localization of FGF-2 is Increased in Cells Plated on Fibronectin

Fibronectin (FN) is an extracellular matrix component known to be associated with injury [Murakami and Otori, 1992], and the nuclear localization of HSPG is increased in corneal stromal fibroblasts (CSF) plated on FN [Richardson et al., 2000]. Because nuclear accumulation of FGF-2 correlates with cell growth, we hypothesized that HSPG might act as a nuclear shuttle for FGF-2 in response to injury. To determine if FGF-2 localizes to the nucleus under the same conditions that HSPG localizes to the nucleus, we plated CSF on FN or collagen I (CO), treated them with ^{125}I-FGF-2, and isolated nuclear fractions. Nuclear FGF-2 in FN-plated cells was consistently higher compared to that in CO-plated cells, and this effect was both time- (Fig. 1A) and dose-dependent (Fig. 1B). Cells plated on FN consistently showed increased nuclear localization of FGF-2 up to 6 h. By 6 h, nuclear levels of FGF-2 began to level off. The dose response study shows that this difference was maintained with increasing concentrations of FGF-2 (Fig. 1B). It is unlikely that this process is related to FGF-2 receptor interactions over this concentration range as the level of FGF-2-bound receptors would be nearly constant ($K_d = 0.02$ nM), while HSPG binding ($K_d = 7.8$ nM) would progressively increase [Richardson et al., 1999]. Thus, it is more likely that the dose response results are reflective of HSPG interactions. Since the nuclear localization of FGF-2 was increased under the same conditions that nuclear HSPG increases, we hypothesize that HSPG is functioning as a shuttle for FGF-2 translocation to the nucleus.

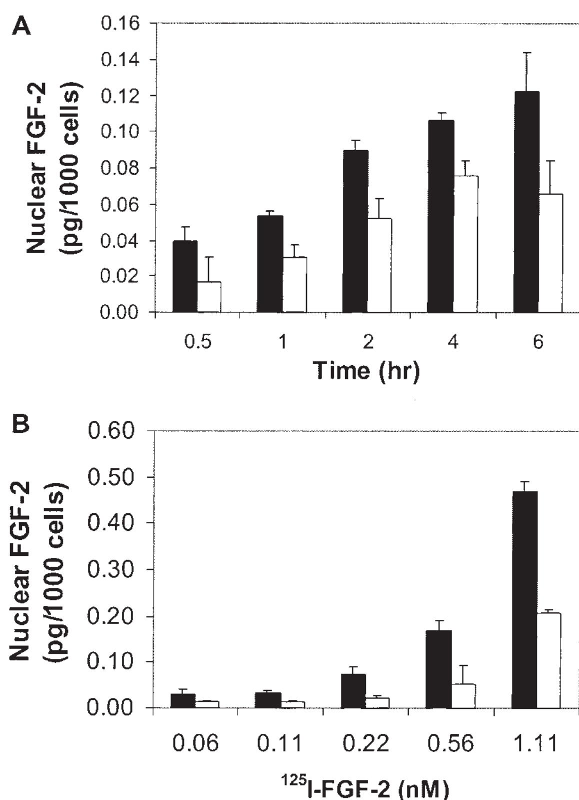


Fig. 1. Nuclear localization of FGF-2 is increased in cells plated on FN vs. CO. Fibroblasts were plated on FN (■) or CO (□) in serum-free media and incubated at 37°C for 16 h. **A**: ^{125}I-FGF-2 (0.6 nM) was added and nuclear fractions were obtained at various time points. **B**: Varying concentrations of ^{125}I-FGF-2 were added and nuclear fractions were obtained following 6-h incubation at 37°C. Data presented are means of triplicates ±1 SD. Similar results were observed in six separate experiments.

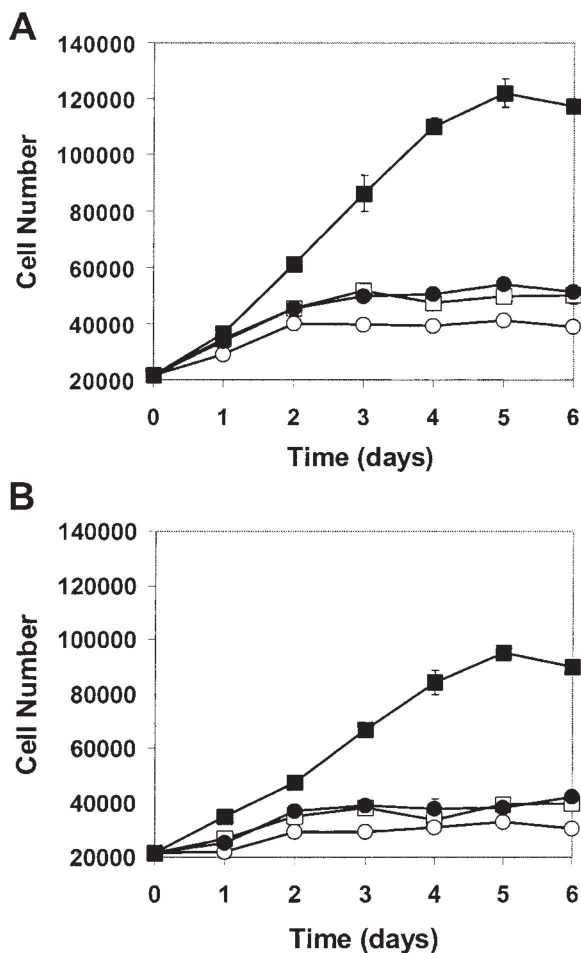


Fig. 2. Cell proliferation is increased on fibronectin and is HSPG-mediated. Cells were cultured in serum-free DMEM on bacteriological plates coated with either fibronectin (**A**), or collagen type I (**B**). Cells were treated daily with heparinase III (0.4 U/ml) or FGF-2 (55 pM). Cell number was determined at each time point using a Coulter counter. Data represent the average of triplicate determinations ± SEM. Control, untreated cells (□); FGF-2 (■); heparinase III (○); FGF-2 and heparinase III (●). These experiments were repeated three times.

Cell Proliferation is Increased on Fibronectin and is HSPG-Mediated

FGF-2 has been shown to bind to casein kinase-2 (CK2) in nuclear extracts and stimulate its activity toward nucleolin [Bonnet et al., 1996]. CK2 signaling in the nuclear matrix is thought to be involved in cell growth and proliferation [Faust and Montenarh, 2000; Yu et al., 2001]. To study the effects of fibronectin on FGF-2-stimulated cell proliferation, cells were plated on FN or CO and treated with FGF-2 in the presence or absence of heparinase III, which cleaves the heparan sulfate chains of HSPG. Cell numbers were determined at various times over a period of 6 days. The results demonstrate that FGF-2-stimulated cell proliferation was increased in cells plated on FN (Fig. 2A) compared to CO (Fig. 2B) by ~30% in the absence of heparinase III. However, in the presence of heparinase III, there was no FGF-2-stimulated proliferation and no significant differences between cells plated on FN vs. CO.

Heparinase III Decreases the Nuclear Localization of FGF-2

FGF-2 is known to interact with HSPG through its heparin-binding domains [Nugent and Iozzo, 2000; Tumova and Couchman, 2000]. To determine if the interaction between FGF-2 and the heparan sulfate chains of HSPG is important for the nuclear translocation of FGF-2,

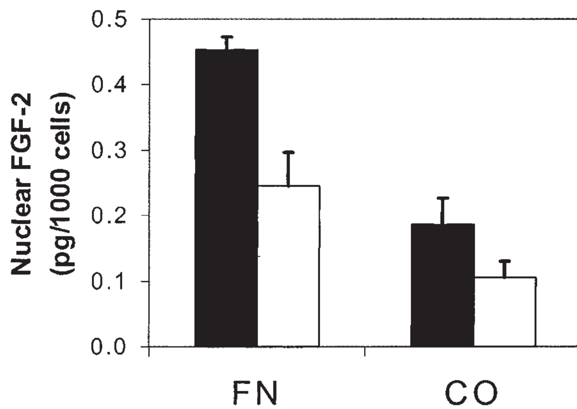


Fig. 3. Heparinase III decreases the nuclear localization of FGF-2 in corneal stromal fibroblasts (CSF) plated on FN and CO. Fibroblasts were plated on FN or CO in serum-free media and incubated at 37°C for 16 h. Following treatment with (□) or without (■) heparinase III (0.2 U/ml) for 1 h at 37°C, cells were rinsed and incubated with ^{125}I-FGF2 (0.6 nM) for 6 h at 37°C in a medium containing an additional dose of heparinase III (0.2 U/ml). Nuclear fractions were isolated and counted in a gamma counter. Data presented are means of triplicates ± 1 SD. Similar results were observed in four separate experiments.

we treated cells with heparinase III, prior to addition of ^{125}I-FGF-2 and nuclear fractionation. Pre-treatment with heparinase III significantly decreased the nuclear accumulation of FGF-2 by over 50% in both FN- and CO-plated cells (Fig. 3), suggesting that the FGF-2-heparan sulfate interaction at the cell surface is necessary for the nuclear translocation of FGF-2.

Nuclear Localization of FGF-2 is Decreased in HS-Deficient CHO cells

The mutant CHO cell line, *ldlD*, is unable to properly synthesize heparan sulfate chains due to a defect in the enzyme, 4-epimerase [Krieger et al., 1989]. This defect in heparan sulfate synthesis can be bypassed by the addition of galactose. We used this CHO cell system to evaluate the requirement of heparan sulfate in the nuclear localization of FGF-2. We plated control (K1) and mutant (ldlD) cells with and without galactose, on FN, treated them with ^{125}I-FGF-2, and isolated nuclear fractions (Fig. 4). Nuclear FGF-2 was decreased in the mutant cells by ~50% compared to control (K1) cells. Moreover, galactose supplementation was able to rescue FGF-2 nuclear localization in the mutant (ldlD) cells back to levels comparable to those observed with control (K1) cells.

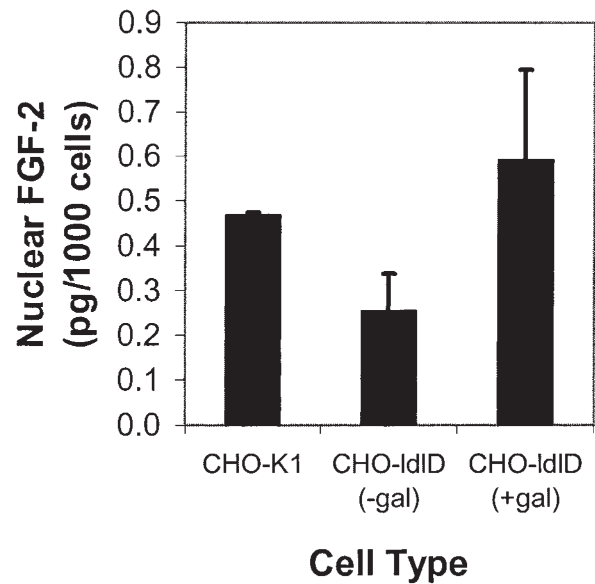


Fig. 4. Nuclear localization of FGF-2 is decreased in HS-deficient CHO-ldlD cells. Wild-type (K1) and HS-reduced (ldlD) CHO cells were plated on FN in serum-free media, 1% ITS, with and without 20 μM galactose and incubated at 37°C for 16 h. ^{125}I-FGF-2 (0.6 nM) was added and the cells were incubated for an additional 6 h at 37°C. Nuclear fractions were isolated and counted in a gamma counter. Data presented are means of triplicates ± 1 SD. Similar results were observed in three separate experiments.

PMA Decreases the Nuclear Localization of FGF-2

Recent publications are beginning to elucidate the roles of signaling through the cytoplasmic tails of syndecans. In particular, syndecan-4 was shown to activate PKCα [Oh et al., 1997b; Rapraeger and Ott, 1998; Horowitz et al., 1999]. To determine if PKC signaling is involved in the nuclear localization of FGF-2, we treated cells with phorbol 12-myristate 13-acetate (PMA), at various time-points relative to FGF-2 introduction. Nuclear fractionation analysis revealed that PMA inhibited the nuclear localization of FGF-2 (Fig. 5A). To measure the amount of activated and total PKCα, we performed Western blot analysis using antibodies specific for phospho-PKCα and total PKCα. This analysis revealed that PMA caused an overall decrease in both phospho- and total PKCα over a 24-h period. Quantitation of relative band intensities revealed that PMA treatment caused greater than 90% loss in total PKCα after 1 h, while phospho-PKCα was decreased by 40–50% after 1 h and by

greater than 90% after 16 h treatment with PMA. This rapid loss in PKCα is consistent with previous reports demonstrating that phorbol ester-mediated activation of PKCα triggers its degradation via the ubiquitin-proteasome pathway [Lu et al., 1998]. The potent down regulatory effects of PMA on PKCα levels suggests that the PMA-mediated decrease in the nuclear localization of FGF-2 might be reflective of the loss of PKCα (Fig. 5B). Interestingly, PMA treatment also decreased the level of nuclear HSPG [Richardson et al., 2000].

PKC Isozymes Regulate the Nuclear Localization of FGF-2

PKCδ was shown to regulate the ability of syndecans to activate PKCα [Murakami et al., 2002]. To further study the role of PKC signaling in the nuclear localization of FGF-2 and to determine if specific PKC isozymes regulate this process, we utilized pharmacological inhibitors for various PKC isozymes. Gö 6976 selectively inhibits Ca^{2+}-dependent PKC isozymes such as PKCα [Martiny-Baron et al., 1993]; rottlerin selectively inhibits PKCδ over other isozymes [Gschwendt et al., 1994]; and bisindolylmaleimide I (Bis I) selectively inhibits PKCα over other isozymes [Wilkinson et al., 1993]. The IC_{50}'s for these inhibitors are presented in Figure 6A. We pre-treated cells with various doses of these inhibitors followed by the addition of ^{125}I-FGF-2 and nuclear fractionation, to elucidate the functions of specific PKC isozymes in the nuclear localization of FGF-2. Treatment with Gö 6976 resulted in a decrease in nuclear FGF-2 past its IC_{50} concentration, suggesting that PKCα activation may be involved in this process (Fig. 6B). In addition, Bis I and rottlerin inhibited the nuclear localization of FGF-2 at certain doses, which likely reflect the differences in the IC_{50}'s for the different PKC isozymes. At low concentrations of Bis I, where PKCα is selectively inhibited over PKCδ, nuclear localization of FGF-2 was decreased by almost 50%, but as the concentration of Bis I was increased to levels where PKCδ would be inhibited, a gradual recovery in nuclear FGF-2 was observed (Fig. 6C). In an opposite manner, at low concentrations of rottlerin, where PKCδ is selectively inhibited over PKCα, nuclear FGF-2 levels were not affected. As the concentration of rottlerin was increased, thereby increasing the inhibition of PKCα, a gradual decrease in nuclear FGF-2 was observed (Fig. 6D). Taken together, these results suggest that the nuclear localization of FGF-2 may involve a mechanism that requires PKCα and is inhibited by PKCδ.

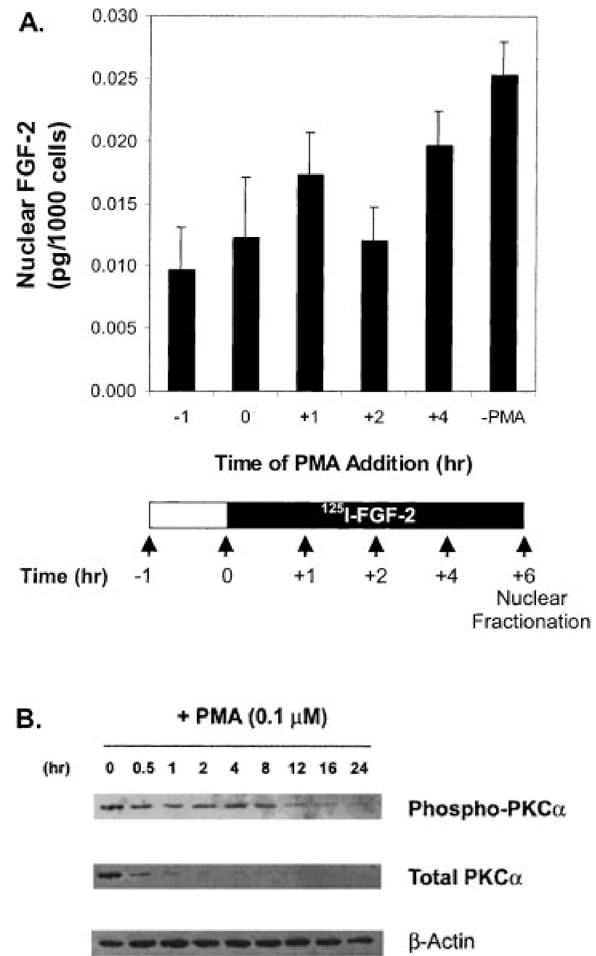
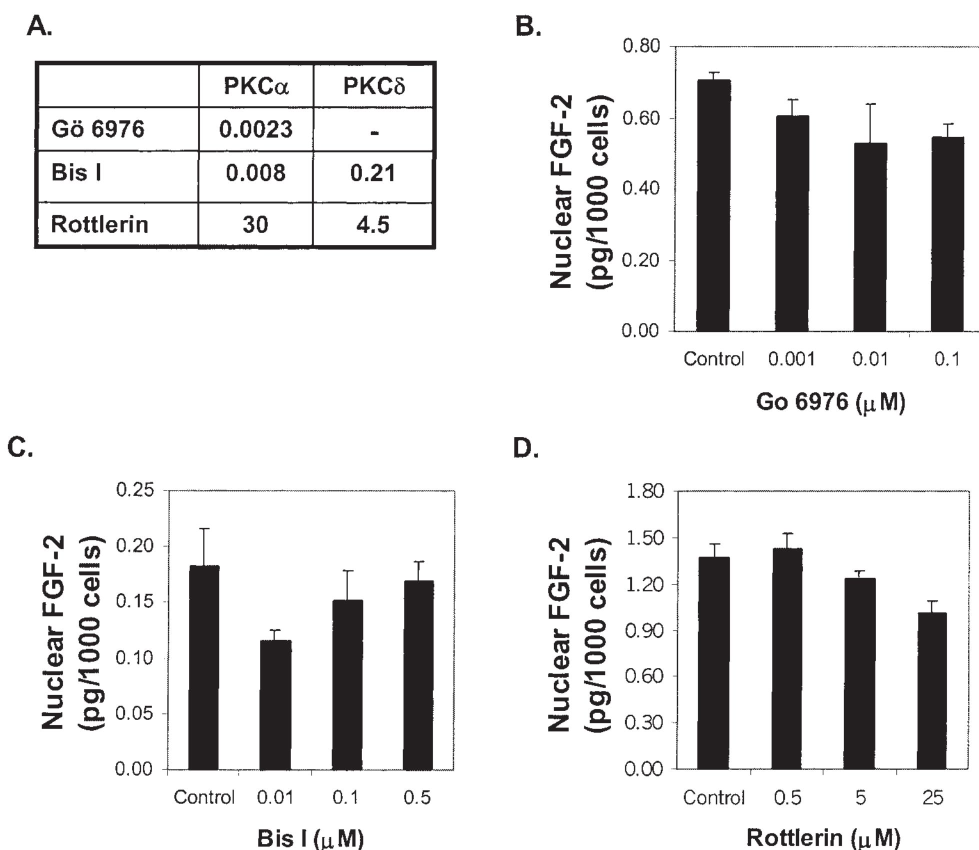


Fig. 5. PMA decreases the nuclear localization of FGF-2. Fibroblasts were plated on FN in serum-free media and incubated at 37°C for 16 h. **A**: PMA (0.1 μM) was added to cells at the indicated times relative to introduction of ^{125}I-FGF-2 (i.e., −1 indicates that PMA was added 1 h prior to ^{125}I-FGF-2). ^{125}I-FGF-2 (0.6 nM) was added at time = 0 and the cells were incubated for 6 h at 37°C. Nuclear fractions were isolated and counted in a gamma counter. Data presented are means of triplicates ± 1 SD. Similar results were observed in three separate experiments. A linear regression analysis was run to determine if there was a relationship between the period of PMA treatment and nuclear FGF levels. The regression was highly significant with $F = 31.1$, $R^2 = 0.64$, $P < 0.001$. The regression coefficient for time was −0.22 which was significantly different than 0.0 with $t = -5.57$ ($P < 0.001$). **B**: Cells were treated with PMA (0.1 μM) for the indicated times at 37°C. Cell extracts were harvested and subjected to 8.5% SDS–PAGE followed by Western blot with antibodies to phospho-PKCα, total PKCα, or β-actin.



A.

	PKCα	PKCδ
Gö 6976	0.0023	-
Bis I	0.008	0.21
Rottlerin	30	4.5

Fig. 6. Different PKC isozymes regulate the nuclear localization of FGF-2. Fibroblasts were plated on FN in serum-free media and incubated at 37°C for 16 h. Following pre-treatment for 1 h with increasing doses of Gö 6976 (**B**), Bis I (**C**), and rottlerin (**D**), ^{125}I-FGF-2 (0.6 nM) was added and cells were incubated for an additional 6 h at 37°C. Nuclear fractions were isolated and counted in a gamma counter. IC_{50} values (μM) for these isozyme-specific PKC inhibitors are shown (**A**). Data presented are means of triplicates ±1 SD. Similar results were observed in three separate experiments. Analysis of variance (ANOVA) followed by the Newman-Keuls multiple comparison t-test was run for each inhibitor. For all three drugs the ANOVA was significant at $P < 0.05$. For Gö 6976, the Newman-Keuls test shows that dose 0.1 μM was significantly different than zero. For Bis I, the Newman-Keuls test shows that dose 0.01 μM was significantly different than zero. For rottlerin, the Newman-Keuls test shows that doses 5 and 25 μM were significantly different than zero.

DISCUSSION

In addition to their cell-surface interactions, FGF-2 and other heparin-binding growth factors are becoming increasingly recognized for their ability to modulate cellular events at the nuclear level [Jans and Hassan, 1998; Keresztes and Boonstra, 1999]. FGF-2 has been shown to bind and activate CK2, an enzyme whose activity in the nucleus correlates with cell proliferation [Bonnet et al., 1996]. The role of FGF-2 in the nucleus may be separate from its traditional role in activating cell surface tyrosine kinase receptors [Bailly et al., 2000]. However, the mechanism by which FGF-2 translocates to the nucleus remains unclear, though HSPG have been identified as potential players [Amalric et al., 1994; Sperinde and Nugent, 1998]. In this report, we investigated the role of HSPG in the nuclear localization of FGF-2. We hypothesize that HSPG function as a shuttle to deliver FGF-2 to the nucleus and that this process is regulated by PKC signaling. We found that both the nuclear localization of FGF-2 and FGF-2-stimulated cell proliferation were increased in cells plated on FN compared to CO, and that these processes were decreased upon treatment with heparinase III. We observed that PMA treatment caused a decrease in both FGF-2 nuclear localization and PKCα levels. In addition, we found that treatment with PKC isozyme-specific pharmacological inhibitors uncovered a potential mechanism in which PKCα might be involved in mediating the

nuclear localization of FGF-2, while PKCδ inhibits it. Thus, in response to injury, FGF-2 may be translocated to the nucleus by HSPG through PKC signaling in order to stimulate cell proliferation of HSPG-expressing cells.

The nuclear localization of FGF-2 was increased in cells plated on FN compared to those plated on CO. Interestingly, the nuclear localization of HSPG was shown to increase in CSF plated on FN compared to those plated on CO [Richardson et al., 2000]. Taken together, these results suggest that HSPG might function as a shuttle for FGF-2 nuclear translocation. In the cornea, the coordinated appearance of HSPG and FN at sites of injury suggest their involvement in the wound healing response [Murakami and Otori, 1992; Brown et al., 1995]. One possibility is that FN induces localized clustering of HSPG on the cell surface that activates the nuclear localization of HSPG-FGF-2 complexes, resulting in increased cell proliferation. Interestingly, we found that FGF-2-stimulated cell proliferation was increased in cells plated on FN compared to CO, suggesting that the nuclear localization of HSPG and FGF-2 are related to cell proliferation. In addition, treatment with heparinase III abrogated these responses, indicating the requirement for FGF-2-heparan sulfate interactions.

Treatment with heparinase III decreased the nuclear localization of FGF-2, demonstrating that interactions with the heparan sulfate chains of HSPG are critical for FGF-2 nuclear translocation. In mutant CHO cells with a defect in the ability to properly synthesize heparan sulfate chains, nuclear localization of FGF-2 was decreased compared to wild-type cells. This effect was rescued by the addition of galactose, which restores the ability for these mutant cells to synthesize heparan sulfate chains. These results address two issues. First, they contribute to the hypothesis for an HSPG-mediated mechanism for the nuclear localization of FGF-2. Second, they suggest that the FGF receptor does not play a major role in this process since CHO cells express extremely low levels of FGF receptor, yet still retained the ability to translocate FGF-2 to the nucleus [Roghani and Moscatelli, 1992].

Among the cell surface HSPG, syndecans contain conserved cytoplasmic tails that have been implicated in initiating intracellular signaling [Tumova and Couchman, 2000]. For example, PKCα becomes activated upon binding to the variable region within the cytoplasmic tail of syndecan-4 [Oh et al., 1997b; Horowitz, 1998a; Oh et al., 1998]. The downstream consequences of PKCα activation are not yet understood. Our data suggests that PKC signaling may regulate the nuclear localization of FGF-2. Treatment with PMA resulted in decreased nuclear localization of FGF-2. Interestingly, PMA was also shown to decrease the nuclear localization of HSPG [Richardson et al., 2000]. Under these conditions, we observed that PMA downregulated both activated and non-activated PKCα, suggesting that the PMA-effect might be a reflection of the loss of PKCα. Moreover, treatment with PKC isozyme-specific pharmacological inhibitors revealed a potential mode of regulation involving PKCα and PKCδ. Treatment with Gö 6976, a PKCα/β inhibitor, resulted in a decrease in nuclear FGF-2 over concentrations that border the IC_{50} for PKCα. Treatment with Bis I at concentrations near its IC_{50} for PKCα (0.008 μM) resulted in decreased nuclear accumulation of FGF-2. However, treatment with increasing concentrations of Bis I resulted in a gradual recovery of nuclear FGF-2, suggesting that as other PKC isozymes, such as PKCδ ($IC_{50} = 0.21$ μM), became inhibited, nuclear localization of FGF-2 was restored. Conversely, treatment with low concentrations of rottlerin, where PKCδ is primarily being inhibited ($IC_{50} = 4.5$ μM), nuclear localization of FGF-2 seemed unaffected. However, treatment with increasing concentrations of rottlerin, where other PKC isozymes, such as PKCα ($IC_{50} = 30$ μM), would be expected to be inhibited, resulted in a gradual decrease in FGF-2 nuclear localization. Taken together, these results suggest a mechanism in which PKCα is involved in mediating the nuclear localization of FGF-2, while PKCδ possibly inhibits it. However, it is important to note that none of the inhibitors showed greater than 50% inhibition of nuclear FGF-2 levels suggesting that while PKC signaling is likely involved, the process may also involve additional pathways. Relating to a possible mechanism for PKC action in this process, PKCδ was shown to phosphorylate a site within the variable region of syndecan-4, resulting in the inhibition of PKCα binding and subsequent activation [Murakami et al., 2002]. Thus, phosphorylation by PKCδ might mark syndecan-4 for cell surface retention, while dephosphorylation and PKCα activation might provide signals for endocytosis and nuclear

translocation. Furthermore, FN increases the production of phosphatidylinositol-4,5-bisphosphate, which is required for the binding and subsequent activation of PKCα by syndecan-4 [McNamee et al., 1993; Horowitz et al., 1999]. This could participate in the increased nuclear localization of FGF-2 in cells plated on FN.

Understanding the processes involved in wound healing could lead to the development of treatment strategies for diseases involving improper tissue repair, such as blindness resulting from corneal scarring. One of these processes could be the HSPG-mediated nuclear localization of cellular regulatory proteins such as FGF-2. It is possible that this process is reflective of a general mechanism whereby other heparin-binding growth factors are transported from the extracellular environment to the nucleus. Even more intriguing is the possibility that HSPG itself might be directly involved in modulating cellular events in the nucleus. Accumulating evidence suggests that the appearance of heparan sulfate in the nucleus may directly regulate proliferative processes [Fedarko et al., 1989; Rykova and Grigorieva, 1998; Cheng et al., 2001]. Moreover, these effects could play a role in a number of pathologies such as in the growth of malignant tumors [Liu et al., 2002a,b], adding a new target to the list for potential cancer treatment strategies.

The ability for heparan sulfate to modulate nuclear processes lies in its complex structure. Chain length and multiple sites for acetylation and sulfation allow HSPG to achieve an enormous degree of variation in structure, which in turn, could lead to specific interactions with a multitude of nuclear proteins [Shriver et al., 2002]. For example, heparin, similar in structure to heparan sulfate, has been shown to inhibit CK2 activity [Hathaway et al., 1980; O'Farrell et al., 1999]. Heparan sulfate has been shown to inhibit DNA topoisomerase I activity [Kovalszky et al., 1998]. Interestingly, these findings might be related, as it has been shown that CK2 and DNA topoisomerase II associate in an active complex [Bojanowski et al., 1993]. Thus, heparan sulfate may inhibit CK2 activity, thereby inhibiting activation of DNA topoisomerases. In addition, HSPG have been shown to bind nucleosomes and participate in chromatin clearance [Du Clos et al., 1999; Watson et al., 1999]. Potentially, the polyanionic nature of heparan sulfate chains could allow them to interact with positively charged lysine groups of histones. These interactions have the potential to impact acetylation processes since acetylation involves the modification of lysine residues. Therefore, heparan sulfate–histone interactions could ultimately impact gene transcription [Gregory et al., 2001].

Our results suggest that the HSPG-mediated nuclear localization of FGF-2 is regulated by PKC signaling and is part of the wound healing response in the corneal stroma. We hypothesize that the activation of PKCα through the cytoplasmic domain of syndecan-4 is assisted by the interaction of FN with HSPG on the cell surface leading to the nuclear localization of HSPG along with heparan sulfate-bound factors such as FGF-2. Thus, through interactions with heparan sulfate chains, FGF-2 is translocated to the nucleus, where it might directly influence cell proliferation by interacting with transcriptional regulators, such as CK2. Further elucidation of the roles of nuclear HSPG would greatly advance the development of effective approaches to treat wound-healing disorders.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Dr. Vickery Trinkaus-Randall, Veronica Kleipeis, and Ling-Ling Yang for assistance in CSF isolation and culture. We also thank Dr. Beth Denholm at Biomarin Pharmaceuticals and Dr. W. Kavanaugh at Chiron for providing heparinase III and FGF-2, respectively. We are grateful to Gerald Coffman from the Boston University School of Public Health for his expert assistance and advice in conducting the statistical analyses of the data presented.

REFERENCES

Amalric FBG, Bonnet H, Brethenou P, Roman AM, Truchet I, Quarto N. 1994. Fibroblast growth factor-2 (FGF-2) in the nucleus: Translocation process and targets. Biochem Pharmacol 47:111–115.

Asthagiri AR, Nelson CM, Horwitz AF, Lauffenburger DA. 1999. Quantitative relationship among integrin–ligand binding, adhesion, and signaling via focal adhesion kinase and extracellular signal-regulated kinase 2. J Biol Chem 274:27119–27127.

Bailly KSF, Leroy D, Amalric F, Bouche G. 2000. Uncoupling of cell proliferation and differentiation activities of basic fibroblast growth factor. FASEB J 14:333–344.

Barcellos-Hoff MH. 2001. It takes a tissue to make a tumor: Epigenetics, cancer, and the microenvironment. J Mammary Gland Biol Neoplasia 6:213–221.

Bojanowski K, Filhol O, Cochet C, Chambaz EM, Larsen AK. 1993. DNA topoisomerase II and casein kinase II associate in a molecular complex that is catalytically active. J Biol Chem 268:22920–22926.

Bonnet HFO, Truchet I, Brethenou P, Cohet C, Amalric F, Bouche G. 1996. Fibroblast growth factor-2 binds to the regulatory β subunit of CK2 activity toward nucleolin. J Biol Chem 271:24781–24787.

Brown CT, Applebaum E, Banwatt R, Trinkaus-Randall V. 1995. Synthesis of stromal glycosaminoglycans in response to injury. J Cell Biochem 59:57–68.

Buczek-Thomas JA, Chu CL, Rich CB, Stone PJ, Foster JA, Nugent MA. 2002. Heparan sulfate depletion within pulmonary fibroblasts: Implications for elastogenesis and repair. J Cell Physiol 192:294–303.

Cheng F, Petersson P, Arroyo-Yanguas Y, Westergren-Thorsson G. 2001. Differences in the uptake and nuclear localization of anti-proliferative heparan sulfate between human lung fibroblasts and human lung carcinoma cells. J Cell Biochem 83:597–606.

Du Clos TW, Volzer MA, Hahn FF, Xiao R, Mold C, Searles RP. 1999. Chromatin clearance in C57Bl/10 mice: Interaction with heparan sulphate proteoglycans and receptors on Kupffer cells. Clin Exp Immunol 117:403–411.

Esko JD, Selleck SB. 2002. ORDER OUT OF CHAOS: Assembly of ligand binding sites in heparan sulfate. Annu Rev Biochem 71:435–471.

Fannon MNM. 1996. Basic fibroblast growth factor binds its receptors, is internalized, and stimulates DNA synthesis in Balb/c3T3 cells in the absence of heparan sulfate. J Biol Chem 271:17949–17956.

Faust M, Montenarh M. 2000. Subcellular localization of protein kinase CK2. A key to its function? Cell Tissue Res 301:329–340.

Fedarko NS, Conrad HE. 1986. A unique heparan sulfate in the nuclei of hepatocytes: Structural changes with the growth state of the cells. J Cell Biol 102:587–599.

Fedarko NS, Ishihara M, Conrad HE. 1989. Control of cell division in hepatoma cells by exogenous heparan sulfate proteoglycan. J Cell Physiol 139:287–294.

Funderburgh JL, Chandler JW. 1989. Proteoglycans of rabbit corneas with nonperforating wounds. Invest Ophthalmol Vis Sci 30:435–442.

Gregory PD, Wagner K, Horz W. 2001. Histone acetylation and chromatin remodeling. Exp Cell Res 265:195–202.

Gschwendt M, Muller HJ, Kielbassa K, Zang R, Kittstein W, Rincke G, Marks F. 1994. Rottlerin, a novel protein kinase inhibitor. Biochem Biophys Res Commun 199:93–98.

Hassell JR, Cintron C, Kublin C, Newsome DA. 1983. Proteoglycan changes during restoration of transparency in corneal scars. Arch Biochem Biophys 222:362–369.

Hathaway GM, Lubben TH, Traugh JA. 1980. Inhibition of casein kinase II by heparin. J Biol Chem 255:8038–8041.

Horowitz A SM. 1998a. Phosphorylation of the cytoplasmic tail of syndecan-4 regulates activation of protein kinase Cα. J Biol Chem 273:25548–25551.

Horowitz A SM. 1998b. Regulation of syndecan-4 phosphorylation in vivo. J Biol Chem 273:10914–10918.

Horowitz A, Murakami M, Gao Y, Simons M. 1999. Phosphatidylinositol-4,5-bisphosphate mediates the interaction of syndecan-4 with protein kinase C. Biochemistry 38:15871–15877.

Horowitz A, Tkachenko E, Simons M. 2002. Fibroblast growth factor-specific modulation of cellular response by syndecan-4. J Cell Biol 157:715–725.

Ingber DE. 1990. Fibronectin controls capillary endothelial cell growth by modulating cell shape. Proc Natl Acad Sci USA 87:3579–3583.

Ishihara M, Fedarko NS, Conrad HE. 1986. Transport of heparan sulfate into the nuclei of hepatocytes. J Biol Chem 261:13575–13580.

Jans DA, Hassan G. 1998. Nuclear targeting by growth factors, cytokines, and their receptors: A role in signaling? Bioessays 20:400–411.

Keresztes M, Boonstra J. 1999. Import(ance) of growth factors in(to) the nucleus. J Cell Biol 145:421–424.

Kovalszky IDJ, Olah-Nagy J, Pogany G, Tovary J, Timar J, Kopper L, Jeney A, Iozzo R. 1998. Inhibition of DNA topoisomerase I activity by heparan sulfate and modulation by basic fibroblast growth factor. Mol Cell Biochem 183:11–23.

Krieger M, Reddy P, Kozarsky K, Kingsley D, Hobbie L, Penman M. 1989. Analysis of the synthesis, intracellular sorting, and function of glycoproteins using a mammalian cell mutant with reversible glycosylation defects. Methods Cell Biol 32:57–84.

Kwan CP, Venkataraman G, Shriver Z, Raman R, Liu D, Qi Y, Varticovski L, Sasisekharan R. 2001. Probing fibroblast growth factor dimerization and role of heparin-like glycosaminoglycans in modulating dimerization and signaling. J Biol Chem 276:23421–23429.

Liu D, Shriver Z, Qi Y, Venkataraman G, Sasisekharan R. 2002a. Dynamic regulation of tumor growth and metastasis by heparan sulfate glycosaminoglycans. Semin Thromb Hemost 28:67–78.

Liu D, Shriver Z, Venkataraman G, El Shabrawi Y, Sasisekharan R. 2002b. Tumor cell surface heparan sulfate as cryptic promoters or inhibitors of tumor growth and metastasis. Proc Natl Acad Sci USA 99:568–573.

Lotti T, Rodofili C, Benci M, Menchin G. 1998. Wound-healing problems associated with cancers. J Wound Care 7:81–84.

Lu Z, Liu D, Hornia A, Devonish W, Pagano M, Foster DA. 1998. Activation of protein kinase C triggers its ubiquitination and degradation. Mol Cell Biol 18:839–845.

Maher PA. 1996. Nuclear translocation of fibroblast growth factor (FGF) receptors in response to FGF-2. J Cell Biol 134:529–536.

Martiny-Baron G, Kazanietz MG, Mischak H, Blumberg PM, Kochs G, Hug H, Marme D, Schachtele C. 1993. Selective inhibition of protein kinase C isozymes by the indolocarbazole Go 6976. J Biol Chem 268: 9194–9197.

McNamee HP, Ingber DE, Schwartz MA. 1993. Adhesion to fibronectin stimulates inositol lipid synthesis and enhances PDGF-induced inositol lipid breakdown. J Cell Biol 121:673–678.

Murakami J, Nishida T, Otori T. 1992. Coordinated appearance of β_1 integrins and fibronectin during corneal wound healing. J Lab Clin Med 120:86–93.

Murakami M, Horowitz A, Tang S, Ware JA, Simons M. 2002. PKC-delta regulates PKC-alpha activity in a syndecan-4 dependent manner. J Biol Chem 277: 20367–20371.

Nugent MA, Edelman ER. 1992. Kinetics of basic fibroblast growth factor binding to its receptor and heparan sulfate

proteoglycan: A mechanism for cooperactivity. Biochemistry 31:8876–8883.
Nugent MA, Iozzo RV. 2000. Fibroblast growth factor-2. Int J Biochem Cell Biol 32:115–120.
O'Farrell F, Loog M, Janson IM, Ek P. 1999. Kinetic study of the inhibition of CK2 by heparin fragments of different length. Biochim Biophys Acta 1433:68–75.
Oh ES, Woods A, Couchman JR. 1997a. Multimerization of the cytoplasmic domain of syndecan-4 is required for its ability to activate protein kinase C. J Biol Chem 272:11805–11811.
Oh ES, Woods A, Couchman JR. 1997b. Syndecan-4 proteoglycan regulates the distribution and activity of protein kinase C. J Biol Chem 272:8133–8136.
Oh ES, Woods A, Lim ST, Theibert AW, Couchman JR. 1998. Syndecan-4 proteoglycan cytoplasmic domain and phosphatidylinositol 4,5-bisphosphate coordinately regulate protein kinase C activity. J Biol Chem 273:10624–10629.
Okada-Ban M, Thiery JP, Jouanneau J. 2000. Fibroblast growth factor-2. Int J Biochem Cell Biol 32:263–267.
Park PW, Reizes O, Bernfield M. 2000. Cell surface heparan sulfate proteoglycans: Selective regulators of ligand-receptor encounters. J Biol Chem 275:29923–29926.
Pellegrini L. 2001. Role of heparan sulfate in fibroblast growth factor signalling: A structural view. Curr Opin Struct Biol 11:629–634.
Quarto N, Amalric F. 1994. Heparan sulfate proteoglycans as transducers of FGF-2 signaling. J Cell Sci 107:3201–3212.
Rapraeger AC. 2000. Syndecan-regulated receptor signaling. J Cell Biol 149:995–998.
Rapraeger AC, Ott VL. 1998. Molecular interactions of the syndecan core proteins. Curr Opin Cell Biol 10:620–628.
Richardson TP, Trinkaus-Randall V, Nugent MA. 1999. Regulation of basic fibroblast growth factor binding and activity by cell density and heparan sulfate. J Biol Chem 274: 13534–13540.
Richardson TP, Trinkaus-Randall V, Nugent MA. 2000. Regulation of heparan sulfate proteoglycan expression and nuclear localization by fibronectin. J Cell Sci 114: 1613–1623.
Roghani M, Moscatelli D. 1992. Basic fibroblast growth factor is internalized through both receptor-mediated and heparan sulfate-mediated mechanisms. J Biol Chem 267:22156–22162.
Rykova VI, Grigorieva EV. 1998. Proteoglycan composition in cell nuclei of mouse hepatoma. Biochemistry (Mosc) 63:1271–1276.
Shriver Z, Liu D, Sasisekharan R. 2002. Emerging views of heparan sulfate glycosaminoglycan structure/activity relationships modulating dynamic biological functions. Trends Cardiovasc Med 12:71–77.
Simons M, Horowitz A. 2001. Syndecan-4-mediated signalling. Cell Signal 13:855–862.
Sperinde GV, Nugent MA. 1998. Heparan sulfate proteoglycans control intracellular processing of bFGF in vascular smooth muscle cells. Biochemistry 37:13153–13164.
Szebenyi GFJ. 1999. Fibroblast growth factors as multifunctional signaling factors. Int Rev Cytol 185:45–106.
Tumova SWA, Couchman JR. 2000. Heparan sulfate proteoglycans on the cell surface: Versatile coordinators of cellular functions. Int J Biochem Cell Biol 32: 269–288.
Turnbull J, Powell A, Guimond S. 2001. Heparan sulfate: Decoding a dynamic multifunctional cell regulator. Trends Cell Biol 11:75–82.
Volk RSJ, Li J, Rosenberg RD, Simons M. 1999. The role of syndecan cytoplasmic domain in basic fibroblast growth factor-dependent signal transduction. J Biol Chem 274: 24417–24424.
Watson K, Gooderham NJ, Davies DS, Edwards RJ. 1999. Nucleosomes bind to cell surface proteoglycans. J Biol Chem 274:21707–21713.
Whitcher JP, Srinivasan M, Upadhyay MP. 2001. Corneal blindness: A global perspective. Bull World Health Organ 79:214–221.
Wilkinson SE, Parker PJ, Nixon JS. 1993. Isoenzyme specificity of bisindolylmaleimides, selective inhibitors of protein kinase C. Biochem J 294:335–337.
Yu S, Wang H, Davis A, Ahmed K. 2001. Consequences of CK2 signaling to the nuclear matrix. Mol Cell Biochem 227:67–71.