The fibroblast, a cell central to effective wound remodeling, not only contains various growth factor receptors but also high activities of a guanylyl cyclase receptor (GC-B). Here we demonstrate that marked elevations of cyclic GMP induced by C-type natriuretic peptide (CNP), the ligand of GC-B, blocks activation of the mitogen-activated protein kinase cascade in fibroblasts. We also show that platelet-derived growth factor, fibroblast growth factor, serum, or Na3VO4 rapidly (within 5 min) and extensively (up to 85% inhibition) disrupt CNP-dependent elevations of cyclic GMP. In addition, the mitogens also lower cyclic GMP concentrations (50% decrease) in cells not treated with CNP. Cyttoplasmic forms of guanylyl cyclase, in contrast to the CNP-stimulated pathway, are not antagonized by the various mitogens. The effects of the mitogens on cellular cyclic GMP are fully explained by a direct and stable inactivation of GC-B. Homogenates obtained from fibroblasts treated with or without the various mitogens contain equivalent amounts of GC-B protein, but both ligand-dependent and ligand-independent activity are markedly (up to 90% inhibition of CNP-dependent activity) decreased after mitogen addition. The stable inactivation is correlated with the dephosphorylation of phosphoserine and phosphothreonine residues of the cyclase receptor. These results not only establish a specific and reciprocal antagonistic relationship between mitogen-activated and GC-B-regulated signaling pathways in the fibroblast but also suggest that one of the earliest events following mitogen activation of a fibroblast is an interruption of cyclic GMP production from this receptor.

Wound healing and tissue remodeling require exquisite spatial and temporal coordination of chemotactic, proliferative, and secretory responses in multiple cells (1). The fibroblast, a cell central to the above processes, is tightly regulated by a host of growth and chemotactic factors that govern its migration, proliferation, and extracellular matrix remodeling (2, 3). A number of years ago we demonstrated that fibroblast cell lines contain particularly high activities of a guanylyl cyclase receptor, GC-B1, that binds C-type natriuretic peptide (CNP) with high affinity (4). CNP, the most highly conserved of the natriuretic peptides (5, 6), is synthesized in various regions throughout the body including endothelial cells but is not found in appreciable quantities in blood, suggesting it acts in an autocrine or paracrine manner. Aside from GC-B, some fibroblast cell lines also appear to contain a soluble form of guanylyl cyclase responsive to nitric oxide (7, 8) and low activities of GC-A, the atrial natriuretic peptide receptor (4).

Substantial evidence exists that cyclic GMP is an antagonist of mitogen action in many cell types. Whether elevated by stimulation of cell-surface receptor-linked guanylyl cyclases, by stimulation of cytosolic guanylyl cyclases, or by direct addition of cell-permeant analogs, cyclic GMP slows the onset of DNA synthesis, decreases cell proliferation, and inhibits chemotaxis (8–14). Thus, significant antagonistic interplay may occur between growth factor-regulated pathways and guanylyl cyclase-regulated pathways in the fibroblast. Here, we demonstrate for the first time that elevations of cyclic GMP block mitogen-induced activation of the MAP kinase pathway in immortalized fibroblasts. We then demonstrate that platelet-derived growth factor (PDGF), fetal bovine serum (FBS), or fibroblast growth factor (FGF) markedly blunt CNP-induced elevations of cyclic GMP in either immortalized fibroblast cell lines or primary fibroblast cultures. The inhibitory effects of the growth factors or of serum on cyclic GMP concentrations are rapid (within 5 min) and extensive and are mediated by a direct and stable inhibition of GC-B. Intriguingly, an inhibition of both ligand-independent and CNP-dependent GC-B activity is evident. The opposing effect of the mitogens is also highly specific, since NO-stimulated elevations of cyclic GMP are not altered by growth factors or serum. The results strongly suggest that a temporal, antagonistic relationship exists between a specific guanylyl cyclase receptor (GC-B) and various mitogens during fibroblast activation and that this occurs in the presence or absence of the ligand, CNP. Since cyclic GMP inhibits the MAP kinase pathway independent of its source of synthesis, the results also suggest that growth factor-induced inhibition of the NO-regulated pathway is not required for mitogen action.

EXPERIMENTAL PROCEDURES

Materials—C-type natriuretic peptide and des-[Cys105,Cys121]atrial natriuretic peptide-(104–126) were from Peninsula Labs; BALB/3T3 (clone A31) and A-10 cell lines were from ATCC; NIH/3T3 cells overexpressing rat guanylyl cyclase-B (GC-B/3T3) were as recently described (14). A BALB/3T3 fibroblast cell line overexpressing the NO-stimulated, heterodimeric (α2/β1) cytosolic guanylyl cyclase was from Dr. Peter Yuen (University of Tennessee, Memphis) and early passage human dermal fibroblasts were from G. Skuta and F. Grinnell (University of Texas Southwestern Medical Center, Dallas, TX). Nucleotides were from Life Technologies, Inc. PDGFβb and basic FGF were from R&D Systems.

‡ To whom correspondence should be addressed: Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX 75235-9050. Tel.: 214-648-5086; Fax: 214-648-5087; E-mail: Ted.Chrisman@email.swmed.edu.

1 The abbreviations used are: GC-B, guanylyl cyclase-B; CNP, C-type natriuretic peptide; GC-A, guanylyl cyclase-A; ANP, atrial natriuretic peptide; FBS, fetal bovine serum; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; IBMX, 1-methyl-3-isobutylxanthine; PAGE, polyacrylamide gel electrophoresis; MAP, mitogen-activated protein; ERK, extracellular signal-regulated kinase; MEK, MAP kinase/ERK kinase; DMEM, Dulbecco's modified Eagle's medium.
Mitogen Regulation of CNP Signaling

Systems. Antibodies to the phosphorylated forms of ERK1/2 and MEK1/2 were from Promega and anti-ERK1 antibodies were from PharMingen. All other reagents were obtained from Sigma unless noted otherwise.

Cell Culture Conditions—Cells were grown and maintained using standard techniques. BALB/C3T3 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) containing antibiotic/antimycotics and 10% calf serum or 10% fetal bovine serum (FBS) as indicated. NIH/3T3 cells overexpressing rat GC-B (GCB/3T3) and BALB/C3T3 cells overexpressing the rat soluble guanylyl cyclase (sGC/3T3) cell lines were maintained in DMEM/antibiotic/antimycotics, 10% FBS, and 0.1 mg/ml G418. Confluent cells were considered quiescent after maintenance in 0.5% FBS for 36–48 h or after 24 h in 0.5% FBS followed by 18–24 h in the absence of FBS.

Intact Cell Studies—Quiescent cells were treated with serum (standard heat-inactivated for tissue culture) and growth factors for varying times 2 to 4 h after adding fresh medium containing the appropriate amount of serum. Vehicle alone or growth factors were added and the cells treated as described in the figure legends. With the exception of FBS, volume additions did not exceed 1% of cell media volume. The cyclic GMP content of intact cells plus medium was determined as follows: IBMX (0.25 mM final) was added and, where indicated, followed 10 min later by CNP (20 nM final) and the cells incubated an additional 10 min. HClO4 (0.5 N final) was added, and the acidified extracts were analyzed for cyclic GMP. Cyclic GMP was estimated by radioimmunoassay following purification of the perchloric acid extracts (16).

Thymidine Incorporation—Quiescent GCB/3T3 fibroblasts (24-well plates) in serum-deprived media were incubated for 1 h with 20 nM CNP followed with 0–1% FBS for 14 h, and then 2 μCi/ml [3H]thymidine (Amersham Pharmacia Biotech) was added for an additional 2 h. The cells were washed with cold phosphate-buffered saline, incubated with 10% trichloroacetic acid for 30 min at 4 °C, washed with 10% trichloroacetic acid, and insoluble material dissolved in 1 N NaOH. Radioactivity in a 50-μl aliquot was determined in a scintillation counter.

Preparation of Cell Homogenates and Estimation of Guanylyl Cyclase Activity—Quiescent cells in 60- or 100-mm dishes were treated with specific growth factors, serum, or Na3VO4, as indicated in the figure legends. The cells were washed twice with cold phosphate-buffered saline, and the dish was immersed in liquid N2 and stored at –80 °C. The frozen cells were thawed at 0–2 °C in 0.3 (60-mm dish) or 0.5 ml of 100 mM homogenization buffer (50 mM Hepes, pH 7.5, 10% glycerol, 100 mM NaCl, 10 μg/ml each of leupeptin, pepstatin, and aprotonin, 50 mM NaF, 1 mM EDTA, and 1 mM Na3VO4) scraped from the dish, and sonicated 3 times for 5 s. Protein concentration (bicinchoninic acid, Pierce) was determined, and the homogenates were aliquoted, frozen in liquid N2, and stored at –80 °C.

Guanylyl cyclase activity was estimated at 37 °C in a final volume of 100 μl. The standard reaction mixture contained, in final concentrations, 50 mM Hepes, pH 7.5, 7.5 mM MgCl2, 1 mM GTP, 1 mM ATP, 120 mM NaCl, 2% glycerol, 0.2 mM EDTA, 10 mM NaF, 1 mM Na3VO4, and 1 mM Na3VO4, CNP, when present, was 20 nM. Maximal guanylyl cyclase activity was estimated under the above conditions with the exceptions that ATP was omitted, MgCl2 was replaced with 5 mM MnCl2, and 1% Triton X-100 was added. The guanylyl cyclase reaction was initiated by the addition of homogenate (5–10 μg of protein) to the prewarmed (37 °C) reaction mixture and terminated by adding 0.5 ml of ice-cold 110 mM Zn(C2H3O2)2 followed by addition of 0.5 ml of 110 mM Na2CO3. The samples were frozen, thawed, and the supernatant fluid (3,000 × g, 15 min) fractionated by alumina chromatography. Cyclic GMP in the eluant was estimated by radioimmunoassay as above. In all cases cyclic GMP formation was measured in linear time and protein concentration.

Western Blot Analysis of Cell Extracts—Following incubations in 6- or 12-well plates, the cells were frozen and thawed in 150 or 300 μl of homogenization medium containing 1% Triton X-100. Detergent-soluble protein was extracted for 1 h on ice, the extracts sonicated, and insoluble material removed by centrifugation for 30 min at 16,000 × g. Proteins were electrophoretically resolved on 8% acrylamide, 0.1% SDS gels and transferred to polyvinylidene fluoride membranes (Immobilon-P, Millipore Corp.). The membranes were probed overnight with antibodies to phosphorylated ERK1/2 (Promega), MEK1/2 (New England Biolabs) or ERK1 (PharMingen) according to the supplier’s instructions. Bound antibodies were detected by chemiluminescence (ECL, Amersham Pharmacia Biotech).

Metabolic Labeling, Immunoprecipitation, and Phosphoamino Acid Analyses—Confluent, quiescent GCB/3T3 cells in 100-mm dishes were incubated 15 h in 5 ml of phosphate-free DMEM containing 1 μCi of [32P]orthophosphate and 0.5% FBS followed by a 1-h incubation in the absence or presence of 10% FBS or 0.1 mM Na3VO4. The cells were washed twice with 5 ml of ice-cold phosphate-buffered saline and frozen on liquid N2. The frozen cells were thawed at 0–2 °C in 0.8 ml of cold homogenization buffer containing 1% Triton X-100, passed through a 25-gauge needle 10–15 times, and rocked at 4 °C for 2 h. The mixture was centrifuged at 170,000 × g for 20 min at 4 °C and the pelleted material discarded. Fifteen μl of normal rabbit serum and 50 μl of a 50% protein A-agarose (Pierce) slurry were added, and the samples were incubated overnight at 4 °C. The protein A-agarose antibody complex was pelleted as above and thoroughly washed in cold homogenization buffer containing 1% Triton X-100. Thirty five μl of Laemmli sample buffer and 3.5 μl of β-mercaptoethanol were added, and the samples boiled and immunoprecipitated GC-B was isolated by SDS-PAGE (0.1% sodium dodecyl sulfate, 8% polyacrylamide). The resolved proteins were transferred to polyvinylidene difluoride membranes and the membrane probed overnight with the same polyclonal antibody as above. Goat anti-rabbit IgG coupled to horseradish peroxidase was visualized by chemiluminescence (Amersham Pharmacia Biotech). Following autoradiography, the bands corresponding to GC-B were excised and phosphoamino acid analyses performed on the acid hydrolysates (17).

RESULTS AND DISCUSSION

CNP Antagonizes Serum Activation of MAP Kinase—Treatment of quiescent GC-B/3T3 fibroblasts with 20 nM CNP prior to

FIG. 1. CNP antagonizes serum-stimulated elevation of phosphorylated ERK1/2. Confluent GCB/3T3 cells were maintained 24 h in DMEM + 0.5% FBS and then 18 h in DMEM alone. Fresh DMEM was added followed in 2 h by addition of FBS to the final concentrations indicated in the figure. Where indicated, the cells were treated with 20 nM CNP for 1 h prior to FBS addition. Cell extracts were prepared and immunoblotted for phosphorylated ERK1/2 (pERK1/2) and total ERK1 (ERK1) as described under “Experimental Procedures.” Each lane represents approximately 6 μg of solubilized extract protein.

FIG. 2. CNP signaling in serum-starved fibroblasts is inhibited by 10% serum. Confluent, serum-starved BALB/C3T3 fibroblasts were switched from 10 to 0.5% FBS or to fresh 10% FBS for 36 h. Then fresh 0.5% FBS (0.5% FBS + 0.5% FBS + 10% + 10+10) was added for h. Following a 10-min incubation with 20 nM CNP and 0.25 μM IBMX, HClO4 was added, and cyclic GMP levels were determined as described under “Experimental Procedures.” Cyclic GMP levels are given as the average of duplicate determinations (± range).
to addition of serum sharply decreased the phosphorylation of ERK1/2 without decreasing the amount of ERK protein (Fig. 1). This is the first observation that activation of a cyclic GMP signaling pathway leads to inhibition of the MAP kinase cascade in fibroblasts. The marked decline in phosphorylated ERK1/2 was accompanied by a decreased phosphorylation of MEK (not shown), suggesting that CNP interferes with an early step in activation of the MAP kinase cascade. It is evident from Fig. 1 that CNP was most effective at low serum concentrations (0–0.3%), less so at intermediate serum concentrations (0.5–1%), and at higher serum concentrations (3–5%) only somewhat effective in blocking ERK phosphorylation. In the absence of serum, treatment of cells with 20 nM CNP for 14 h decreased [3H]thymidine incorporation by an average of 18% and consistent with serum antagonism of the CNP effects on ERK1/2 phosphorylation, 20 nM CNP decreased thymidine incorporation 10% (0.1% serum), about 7% (0.5% serum), and ineffectively at higher amounts of serum (not shown).

The natriuretic peptide clearance receptor (which binds natriuretic peptides but does not possess guanylyl cyclase activity (20)) has been reported to mediate ANP inhibition of mitogen activation of the MAP kinase cascade in some (21) but not all (22) cell lines. To determine if the above effects of CNP were mediated by the clearance receptor in the GC-B/3T3 cell line, des-[Cys105,Cys121]ANP, a ligand selective for the clearance receptor (23), was tested on these cells at concentrations of 20–1000 nM and did not inhibit basal or serum-stimulated ERK1/2 phosphorylation or DNA synthesis. These observations and the ability of low CNP concentrations to inhibit ERK1/2 phosphorylation (Fig. 1) in cells overexpressing GC-B (conditions which would favor CNP acting through GC-B rather than

---

3 Under these conditions, we were unable to detect the presence of mitogen-activated protein kinase phosphatase-1, the MAP kinase phosphatase reportedly induced by ANP in mesangial cells (18).

4 Others (19) also have noted that NIH/3T3 cell lines are relatively resistant to antimitogenic effects of natriuretic peptides, possibly explained by rapid homologous desensitization of GC-A or GC-B (15).
the clearance receptor) are clear evidence that the effects of CNP are mediated by GC-B.

The diminished effectiveness of CNP at the higher serum concentrations could be explained by serum antagonism of CNP signaling. If so, then net signaling by CNP- and serum-stimulated pathways may reflect a balance between these two opposing signaling systems. Experiments on intact and broken cells were thus designed to determine if serum and defined mitogens interfere with CNP signaling (as measured by cyclic GMP elevation in intact cells).

Serum Antagonizes CNP Elevations of Cyclic GMP.—The addition of serum (10%) to quiescent BALB/3T3 fibroblasts (0.5% serum) for 1 h decreased CNP-stimulated elevations of cyclic GMP by nearly 70% (Fig. 2, 0.5+10), whereas the CNP response was unaffected in normally cycling cells (10% serum) (Fig. 2, 10+10). The 25% decline in CNP-elevated cyclic GMP levels in serum-starved cells compared with control cells (Fig. 2, 0.5+0.5 versus 10+10) is not due to a decreased sensitivity to CNP (data not shown) but reflects partial cell loss during serum starvation and possibly decreased expression of GC-B and/or other proteins necessary for signaling.

PDGF Is a Potent Suppressor of CNP Signaling—PDGF, at concentrations of 300–500 pM, is the primary fibroblast mitogen in serum, accounting for at least 50% of the mitogenic activity (24–26). PDGF binding to specific heterodimeric or homodimeric cell-surface receptor tyrosine kinases results in activation of the MAP kinase cascade, protein kinase C, and several other distinct signaling pathways (27–29). Low concentrations of PDGF (in the presence of 0.5% serum) rapidly and effectively interfered with CNP signaling (as monitored by elevation of cyclic GMP) in quiescent BALB/3T3 fibroblasts (Fig. 3). PDGF inhibition was concentration-dependent (Fig. 3A) and, in sub-nanomolar amounts, was as effective as serum in inhibiting CNP signaling. These concentrations of PDGF are well within the range that is mitogenic for this and other cells of mesenchymal origin (30–32). The inset of Fig. 3A shows, by Western blot analysis of phosphorylated ERKs 1 and 2, that concentrations of PDGF that decrease CNP signaling also cause near-maximal activation of the MAP kinase pathway. The inhibitory effect of PDGF developed rapidly (short lag-time) (Fig. 3B) as is the case with PDGF activation of the MAP kinase cascade (33). Treatment of the quiescent cells with 0.3 nM PDGF (in the presence of 0.5% serum) resulted in a rapid (evident within 5 min) and sharp decline in CNP-stimulated elevation of cyclic GMP levels reaching near-maximal inhibition within 60 min. The rapidity of PDGF inhibition is clear from the inset where PDGF was added shortly after CNP. The similar time courses and concentration dependence of PDGF inhibition of the CNP signaling pathway and stimulation of the MAP kinase cascade suggest that suppression of CNP signaling through cyclic GMP is an early downstream consequence of PDGF receptor activation.

That this inhibition of CNP by serum and defined mitogens is not confined to immortalized cell lines was confirmed in rat aortic smooth muscle cells (A-10) and early passage human dermal fibroblasts (not shown). Such results suggest that inactivation of GC-B by mitogens represents a general consequence of growth factor signaling serving to limit cyclic GMP antagonism of growth factor-regulated cell function.

Nitric Oxide Signaling through Cyclic GMP Is Unaffected by Mitogens—The above experiments established that serum and PDGF decrease the cyclic GMP signal generated in response to CNP stimulation of the cell-surface receptor guanylyl cyclase, GC-B. NO and NO-sensitive cytosolic guanylyl cyclase, a key signaling pathway in many cell types (34), have been reported as antimitogenic under some conditions (8, 9) and, as with the

---

**FIG. 4.** Serum and mitogens do not inhibit NO stimulation of soluble guanylyl cyclase in quiescent BALB/3T3 cells. Cells, over-expressing rat α/β2 soluble guanylyl cyclase, were incubated in the absence or presence of 10% FBS, 0.3 nM PDGF, or 0.6 nM basic FGF for 30 min prior to a 10-min incubation with 0.25 mM IBMX then vehicle (−), 20 nM CNP, or 100 μM sodium nitroprusside (SNP) were added for an additional 10 min to control wells (CNP or SNP) or to those with FBS, PDGF, or SNP (+ CNP or + SNP as indicated by the horizontal arrows). Cyclic GMP contents of HClO4 extracts of duplicate wells were determined as given under “Experimental Procedures.” Cyclic GMP levels are given as the average (± range).

**FIG. 5.** Na3VO4 is a potent inhibitor of CNP signaling in serum-starved fibroblasts. Quiescent GCB/3T3 fibroblasts were incubated 30 min with the indicated concentrations of Na3VO4 followed by a 10-min incubation with 20 nM CNP and 0.25 mM IBMX. HClO4 was added and cyclic GMP accumulation determined as given under “Experimental Procedures.” Values for cyclic GMP are means (± S.E.) of triplicate determinations. Inset, quiescent cells were incubated for 15 min with the indicated amounts of Na3VO4 and 6 μg protein analyzed for phosphorylated ERK1/2 (pERK1/2) by immunoblotting as in Fig. 3.
of mitogen signaling (38). Na3VO4, a cell permeant, non-selective protein-tyrosine-phosphatase inhibitor mimics the effects of many ligands that activate protein-tyrosine-kinases and the MAP kinase pathway (39–41). This phosphatase inhibitor also mimics the effects of serum and PDGF on CNP signaling in fibroblasts (Fig. 5). Treatment of quiescent GC-B/3T3 fibroblasts with 5–50 μM Na3VO4 for 50 min reduced elevations of cyclic GMP 50–80%, implying stable inactivation of guanylyl cyclase and providing a mechanism for reversion of ligand-stimulated dephosphorylation and thus dephosphorylation of GC-B (15, 42). Specific phosphoamino acids in GC-B have been identified as necessary for ligand-induced signaling and un-dergo ligand-stimulated dephosphorylation, and thus dephosphorylation of one or more of these residues may account for

Fig. 6. Serum and Na3VO4 treatment of serum-starved fibroblasts results in a rapid and stable inhibition of ligand-dependent and CNP-independent guanylyl cyclase activity.

Basal and CNP-stimulated activities of GC-B in cell homogenates are not inhibited by 1 mM Na3VO4 or by 10% FBS suggesting that their effects in intact cells are not due to direct inhibition of the cyclase. 

The same results were seen with washed 100,000×g pellets.
mitogen-induced inactivation of GC-B (43). The results of the preceding studies show that CNP markedly elevates cyclic GMP in both quiescent (serum-limited) and normal cycling (serum-replete) cells (Fig. 2). However, relatively high serum or defined growth factors added to quiescent cells rapidly and sharply decreases CNP stimulation of GC-B in intact cells (Figs. 2 and 3) by directly lowering cyclase activity (Fig. 6) in the absence of changes in GC-B expression (Fig. 7). It is clear then that mitogens substantially disrupt signaling through inactivation of GC-B, consequently suppressing CNP elevation of cyclic GMP in whole cells or in broken cells. Conversely, CNP antagonism of serum activation of the MAP kinase cascade (Fig. 1) demonstrates that CNP and mitogens are antagonists at least in the “resting” or G0/early G1 phase of the cell cycle. Data in Fig. 2 also imply that serum or mitogen inhibition of CNP signaling is acute in that it therefore appears reversible under chronic conditions.

*Adaptation to Mitogens—*Reversible changes in signaling pathways are important, and since fibroblasts in the proximity of a wound are continuously exposed to high levels of mitogens (1), it is important to determine the effects of such conditions on CNP responsiveness. Basal, CNP-stimulated, and Mn2+ /Triton-stimulated guanylyl cyclase activities in homogenates were determined at different times during an 8-h exposure of quiescent GC-B/3T3 fibroblasts to 10% serum (Fig. 8). Basal and CNP-stimulated guanylyl cyclase activities sharply decreased during the initial 1 h of serum treatment but recovered to control levels by 6 h despite the continued presence of serum. The ability of CNP to elevate cyclic GMP in intact cells (not shown) and the phosphorylation state of the cyclase (not shown). This is consistent with covalent regulation of natriuretic peptide receptor-guanylyl cyclases through phosphorylation previously seen in broken cells (44). Although CNP responses changed with time, both total guanylyl cyclase activity, as measured in the presence of Mn2+ /Triton, and the expression level of the cyclase remained constant. The cyclase therefore appears to be reversibly regulated by covalent modification catalyzed by one or more protein kinases/phosphoprotein phosphatases, at least some of which are mitogen-sensitive. Following rapid serum inactivation of the cyclase CNP signaling is reestablished prior to the onset of DNA synthesis and mitosis,9 coinciding with the initiation and decline of immediate early gene transcription (30). As the cell exits mitosis and is again sensitive to extracellular mitogens,10 it again responds to CNP. The simultaneous, similar, and consistent but opposite effects of serum, PDGF, and Na2VO4 on CNP- and mitogen-signaling pathways strongly suggest that suppression of CNP signaling and activation of the MAP kinase cascade are functionally linked.

Rapid changes in mitogen levels likely occur at the site of a wound as platelets release large amounts of mitogenic/chemoattractive factors such as PDGF. In this context, where fibroblasts are attracted to the wound or stimulated to proliferate, signaling pathways antagonistic to proliferation or migration are likely suppressed. Clearly the acute suppression of GC-B ligand-dependent as well as CNP-independent activity by mitogens is an early event in their signaling pathways. The reversible nature of the inhibition is also physiologically important, but the mechanism of reversibility (mitogen receptor desensitization or signaling pathway component desensitization) remains unknown.

**REFERENCES**

1. Clark, R. A. F. (1995) in *The Molecular and Cellular Biology of Wound Repair*; (Clark, R. A. F., ed) 2nd Ed., pp. 3–50, Plenum Publishing Corp., New York
2. Martin, P. (1997) *Science* 276, 75–81
3. Diegelmann, R. F. (1997) *J. Urol.* 157, 298–302
4. Chirman, T. D., Suh, S., Potter, L. L., and Garbers, D. L. (1993) *J. Biol. Chem.* 268, 3698–3703
5. Tawaragi, Y., Fuchimura, K., Tanaka, S., Minamino, N., Kangawa, K., and Matsuo, K. (1991) *Biochem. Biophys. Res. Commun.* 175, 645–651
6. Suzuki, R., Takahashi, A., and Takei, Y. (1992) *J. Endocrinol.* 135, 317–323
7. Clementi, E., Sciorati, C., Riccio, M., Milose, M., Meldolesi, J., and Nistico, G. (1995) *J. Biol. Chem.* 270, 22277–22282
8. Calderone, A., Thaik, C. M., Takahashi, N., Chang, D. L., and Coluci, W. S. (1998) *J. Clin. Invest.* 101, 812–818
9. Takizawa, T., Gu, M., Chobanian, A. V., and Brecher, P. (1997) *Hypertension* 30, 1035–1040
10. Haneda, M., Araki, S.-I., Sugimoto, T., Togawa, M., Koya, D., and Kikkawa, R. (1996) *Kidney Int.* 50, 384–391
11. Kohno, M., Yokokawa, K., Minami, M., Ueda, M., and Yoshikawa, J. (1997) *Circ. Res.* 81, 585–590
12. Sugimoto, T., Haneda, M., Togawa, M., Isono, M., Minami, N., Kangawa, K., and Kikkawa, R. (1996) *J. Biol. Chem.* 271, 547–554
13. Yu, S.-M., Hung, L.-M., and Lin, C.-C. (1997) *Circulation* 95, 1296–1277
14. Hutchinson, H. G., Trindale, P. T., Cunanan, D. B., Wu, C.-F., and Pratt, R. E. (1997) *Cardiovasc. Res.* 35, 158–167
15. Potter, L. R. (1998) *Biochemistry* 37, 2422–2429
16. Domino, S. E., Tubb, D. L., and Garbers, D. L. (1991) *Methods Enzymol.* 201, 345–355
17. Boyle, W. J., van der Geer, P., and Hunter, T. (1991) *Methods Enzymol.* 201, 110–149
18. Sugimoto, T., Kikkawa, R., Haneda, M., and Shigeta, Y. (1993) *Biochem. Biophys. Res. Commun.* 195, 72–78
19. Appel, R. G. (1990) *Annu. Rev. Pharmacol.* 30, 395–418
20. Fether, J., Meloche, S., Nguyen, T. T., On, H., and de Lean, A. (1989) *Mol. Pharmacol.* 35, 584–594
21. Primo, L., Weber, M. J., Hu, R.-M., Pieman, A., Daniels, M., and Levin, E. R. (1996) *J. Biol. Chem.* 271, 11456–11462
22. Hutchinson, H. G., Trindale, P. T., Cunanan, D. B., Wu, C.-F., and Pratt, R. E. (1997) *Cardiovasc. Res.* 35, 158–167
23. Calhut, P. A., and Hassid, A. (1991) *Biochem. Biophys. Res. Commun.* 179, 1606–1613
24. Grainger, D. J., Moseley, D. E., Metcalf, J. C., Weissberg, P. L., and Kemp, P. R. (1995) *Clin. Chim. Acta* 235, 11–31
25. Kohler, N., and Lipton, A. (1974) *J. Biol. Chem.* 249, 72–78
26. Meyer-Wilkins, W. D., and Eisler, J. (1985) *Cell Biol. Int. Rep.* 9, 389–398
27. Claesson-Welsh, L. (1996) *Biochem. Biophys. Res. Commun.* 238, 373–385
28. Vignais, M.-L., Sadowski, H. B., Watling, D., Rogers, N. C., and Gilman, A. G. (1996) *Mol. Cell. Biol.* 16, 1759–1769
29. Choudhury, G. G., Ghosh-Choudhury, N., and Abboud, H. E. (1998) *J. Clin. Invest.* 12, 2751–2760

---

* In Balb/C3T3 fibroblasts “early G1” ends at 6 h and S phase begins about 12 h after PDGF exposure (30, 45).

10 As cells pass the restriction point in G1, they become refractory to extracellular mitogens presumably regaining full mitogen sensitivity upon completion of the cell cycle (46).
30. Pledger, W. J., Stiles, C. D., Antoniades, H. N., and Scher, C. D. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 4481–4485
31. Lau, L. F., and Nathans, D. (1985) EMBO J. 4, 3145–3151
32. Graf, K., Xi, X.-P., Yang, D., Fleck, E., Haseh, W. A., and Law, R. E. (1997) Hypertension 29, 334–339
33. Dudley, D. T., Pang, L., Decker, S. J., Bridges, A. J., and Saltiel, A. R. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 7686–7689
34. Drewett, J. G., and Garbers, D. L. (1994) Endocrinology 15, 135–162
35. Nesbitt, J. A., III, Anderson, W. B., Miller, Z., Pastan, I., Russell, T. R., and Gospodarowicz, D. (1976) J. Biol. Chem. 251, 2344–2352
36. Garg, U. C., and Hassid, A. (1991) J. Biol. Chem. 266, 9–12
37. LaVallee, T. M., Prudovsky, I. A., McMahon, G. A., Hu, X., and Maciag, T. (1996) J. Cell Biol. 141, 1647–1658
38. Hunter, T. (1995) Cell 80, 225–236
39. Gordon, J. A. (1991) Methods Enzymol. 201, 477–482
40. Posner, B. I., Faure, R., Burgess, J. W., Bevan, A. P., Lachance, D., Zhang-Sun, G., Fantus, I. G., Ng, J. B., Hall, D. A., and Lum, B. S. (1994) J. Biol. Chem. 269, 2596–4604
41. Zhao, Z., Tan, Z., Dilts, C. D., You, M., and Fischer, E. H. (1996) J. Biol. Chem. 271, 22251–22255
42. Potter, L. R., and Garbers, D. L. (1992) J. Biol. Chem. 267, 14531–14534
43. Potter, L. R., and Hunter, T. (1998) J. Biol. Chem. 273, 15533–15539
44. Foster, D. F., and Garbers, D. L. (1998) J. Biol. Chem. 273, 16311–16318
45. Dong, F., Cress, W. D., Jr., Agrawal, D., and Pledger, W. J. (1998) J. Biol. Chem. 273, 6190–6195
46. Sherr, C. J. (1996) Science 274, 1672–1677
Reciprocal Antagonism Coordinates C-type Natriuretic Peptide and Mitogen-signaling Pathways in Fibroblasts
Ted D. Chrisman and David L. Garbers

J. Biol. Chem. 1999, 274:4293-4299.
doi: 10.1074/jbc.274.7.4293

Access the most updated version of this article at http://www.jbc.org/content/274/7/4293

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 45 references, 22 of which can be accessed free at http://www.jbc.org/content/274/7/4293.full.html#ref-list-1