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Localization of Fos and Jun Proteins in Rat Aortic Smooth Muscle Cells after Vascular Injury

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The availability of specific reagents to measure gene activity has provided important tools and potential new directions for the study of smooth muscle cell (SMC) proliferation* in vivo. *In this report, we have measured steady-state mRNA levels of several* fos *and jun family members in aortic tissue by Northern blotting after vascular injury. In addition, protein products of these genes were analyzed by immunocytochemistry. Within 15 minutes of balloon injury, mRNA levels of* c-fos, fosB, c-jun, junB, *and* junD *were elevated severalfold. In contrast,* fos-related antigen (fra-1) *mRNA showed a delayed onset of expression. The expression kinetics of these immediate early genes was similar to those in cultured cells stimulated to undergo proliferation by growth factors, suggesting that such SMC gene activation* in vivo *reflects permeation of blood-derived growth factors into the vessel wall or intravascular release of preformed growth factors. Translation of* fos *and* jun *genes into immunoreactive products was demonstrated 2 hours after balloon injury with antisera to Fos and Jun proteins. Treating rats with cycloheximide abolished this immunoreactivity. The distribution of Fos and Jun products was concentrated in SMC nuclei at the luminal border of the rat aorta. Such focal expression may have consequences for the initiation of SMC DNA synthesis and migration after vascular injury. Furthermore, the expression of Fos and Jun proteins in SMC after vascular balloon injury may be used as an index of SMC activation under a variety of experimental settings. (Am J Pathol 1993, 142:715–724)

Blood vessel balloon de-endothelialization (BDE) evokes a series of events including permeation of platelet factors into the vessel wall[1], initiation of smooth muscle cell (SMC) DNA synthesis[2,3] and SMC migration to and proliferation in the intima of the injured vessel.[4] Such changes in the vessel wall are thought to play an important role in restenosis after angioplasty and bypass graft surgery.[5,6] Elucidating early alterations in SMC gene expression may provide insight into the mechanisms associated with these events. However, there is a paucity of information on the immediate changes in SMC gene expression that ensue after BDE.[7,8]

Immediate early gene (IEG) induction is a common occurrence after growth factor stimulation of quiescent cells in culture[9] and has been demonstrated in several *in vivo* models of cell proliferation.[7,10,11] A large class of IEG encodes transcription factors such as the Fos and Jun families.[12] These molecules are implicated in growth control[13] and function by forming dimers that bind the AP-1–responsive element located in the 5′ promoter region of many genes.[14] Some AP-1 responsive genes, such as transforming growth factor-β1,[15] may control SMC growth and extracellular matrix organization after vessel injury.[16,17] Thus, identifying and localizing cells expressing Fos and Jun proteins *in vivo* may reveal pertinent information about the changes in SMC phenotype subsequent to IEG induction.

Although Fos and Jun proteins have been documented in numerous *in vivo* systems,[18–20] only one preliminary report describes such expression in an *in vivo* model of cell proliferation.[21] In the present study, mRNA expression of several *fos* and *jun* family members is demonstrated in the rat aorta within minutes after BDE. Immunoreactive Fos and Jun proteins are shown to predominate in SMC nuclei closest to the lumen of the vessel 2 hours after BDE. These data

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provide an early molecular picture of SMC activation *in vivo* and suggest that immediate early proteins such as Fos and Jun participate in the initial signalling leading to SMC growth and migration after vascular injury.

Materials and Methods

Animals

Fifty male Sprague-Dawley rats (350 to 375 g) were purchased from Charles River Laboratories (Wilmington, MA) and allowed to acclimate 3 days before experimentation. Food and water were provided *ad libitum*. Animals were handled in accordance with National Institutes of Health and institutional guidelines.

BDE

Rats underwent BDE of the thoracic aorta as described.[22] For RNA studies, at least three rats were pooled in two independent experiments. For immunocytochemistry, three rats underwent BDE and two rats served as non-BDE controls. In some experiments, rats received an injection of 7.5 mg/kg cycloheximide (CHX) immediately after BDE to determine whether protein synthesis inhibition blocks Fos and Jun immunoreactivity.

RNA Isolation/Northern Blotting

Total RNA from whole aortic tissue was isolated 0, 0.25, 0.5, 2, 6, 12, and 24 hours after BDE. Vessels were rapidly excised from the animal at the indicated time, rinsed in chilled PBS, stripped of peri-aortic fat, and flash-frozen in liquid nitrogen. The interval of time from aortic excision to liquid nitrogen was less than 1 minute. Processing of whole aortic tissue RNA for Northern blotting and densitometric scanning was exactly as described.[7]

Immunocytochemistry

Animals underwent BDE (in the absence or presence of CHX) and were killed 2 hours later under sodium pentobarbital (50 mg/kg, intraperitoneally) by cardiac puncture. The aorta was perfused with 50 ml of PBS (pH 7.4), followed by 60 ml of a 4% paraformaldehyde solution (pH 4.0) containing 2% zinc salicylate (City Chemicals, New York, NY) as described.[23] The aorta was gently excised and immersion fixed for 1 hour. After immersion fixation, the aorta was cryoprotected in 30% sucrose/PBS at 4 C for at least 3 days. Cross-sections from the thoracic aorta of non-BDE control and 2-hour BDE animals were embedded in Tissue-Tek II O.C.T. compound Cat. Number 4583 (Miles Laboratories, Milwaukee, WI). Sections (20 μm) were made with a cryostat and immediately placed in a 24-well plate containing 0.5 ml of 0.5 mol/L Tris-HCl (pH 7.6). Sections were equilibrated with buffer by washing for 10 minutes in three successive changes of Tris-HCl buffer. To reduce nonspecific binding of antibodies, sections were exposed to normal goat serum containing 0.5% Triton X-100 for 1 hour before incubating with primary antisera. Quenching endogenous peroxidase was not done because this procedure reduces the specificity of the Fos and Jun antisera (Linda Robertson, personal communication).

All antisera were diluted to 1:2000 in 0.5 mol/L Tris-HCl (pH 7.6) containing 1% normal goat serum and 0.1% Triton X-100. Incubations with primary antisera or control IgG were carried out in humidified 24-well plates at 4 C for 7 days with gentle agitation. Floating sections were washed three times in Tris-HCl buffer and then processed for immunoperoxidase detection using the avidin-biotin complex method (Vector Laboratories, Burlingame, CA) according to the manufacturer's instructions. Stained sections were mounted in 10% glycerol/PBS and coverslipped for microscopy. Some sections were lightly counterstained with hematoxylin to confirm nuclear staining. Brain sections from rats treated with metrazole[18] were kindly provided by Dr. Tom Curran (Roche Institute of Molecular Biology, Nutley, NJ) and served as positive control tissue for Fos and Jun protein staining. Normal rat brains served as negative controls. Aortic sections were viewed with a Nikon microscope using a Zeiss (Tarrytown, NY) 630× lens. Color micrographs were taken with Kodak 200 Gold film and black and white micrographs were obtained using Kodak T-MAX 100 film (Rochester, NY).

Antisera and cDNA Probes

Rabbit antisera to murine Fos and FosB, a 1.8-kb fragment of a murine c-*fos*/v-*fos* fusion gene in pSP64 and a 1.5-kb insert of the rat *fra*-1 in pSP64 were generously given by Dr. Tom Curran. Rabbit antisera to murine Jun and purified inserts to murine *fosB*, *junB*, and *junD* were kindly provided by Dr. Rodrigo Bravo (Bristol-Meyers Squibb Pharmaceutical Research Institute, Princeton, NJ). A 1.9-kb fragment of murine c-*jun* in pGEM was a generous gift from Dr. Lynn Ransone (Salk Institute, La Jolla, CA). A 0.4-kb fragment of v-*Ha-ras* was kindly supplied by

Dr. Judith Campisi (Lawrence Berkeley Laboratories, Berkeley, CA). The 18S rRNA probe was a gift from Dr. Ester Sabban (New York Medical College, Valhalla, NY). The c-*fms*, c-*myb* and β-actin cDNA probes were purchased from ATTC (Rockville, MD) and Oncor Inc. (Gaithersburg, MD).

Results

Aortic fos *Family Gene Expression After BDE*

Aortic SMC show elevated c-fos mRNA after BDE.[7] The latter study employed an enzyme digestion method to separate media from adventitia.[24] Such a procedure elicits c-*fos* expression in non-BDE SMC because of the physical handling of aortae during enzyme digestion.[7] To ascertain the expression pattern of c-*fos* and other *fos* members after BDE independent of *ex vivo* manipulations, total RNA from whole aortic tissue was isolated and sequentially hybridized to c-*fos*, *fra*-1, and *fosB*. Control non-BDE whole aortic tissue showed little *fos* family mRNA expression (Figure 1). However, levels of c-*fos* mRNA increased more than 20-fold 15 minutes after BDE. Such levels were observed 30 minutes after vascular injury before decreasing sharply by 2 hours. A consistent reactivation of c-*fos* transcripts was apparent between 6 and 12 hours post-BDE. By 24 hours, levels of c-*fos* mRNA diminished, but remained slightly elevated over baseline. The onset of *fosB* expression was similar to c-*fos*, but the signal intensity was much less (Figure 1). Levels of *fosB* were undetectable by 6 hours, showing no reactivation as observed with c-*fos*. In contrast to c-*fos* and *fosB*, the onset of *fra*-1 mRNA induction in whole aortic tissue was delayed (Figure 1). Levels of *fra*-1 mRNA increased 2 hours after BDE and then gradually declined toward controls.

Aortic jun *Family Gene Expression after BDE*

Since the proteins encoded by fos family genes form dimers with proteins encoded by *jun* mRNA molecules,[12,25] steady-state transcripts of c-*jun*, *junB*, and *junD* were measured in whole aortic tissue following BDE. In contrast to *fos* members, non-BDE whole aortic tissue showed slight detection of c-*jun* and a prominent signal corresponding to *junD* (Fig-

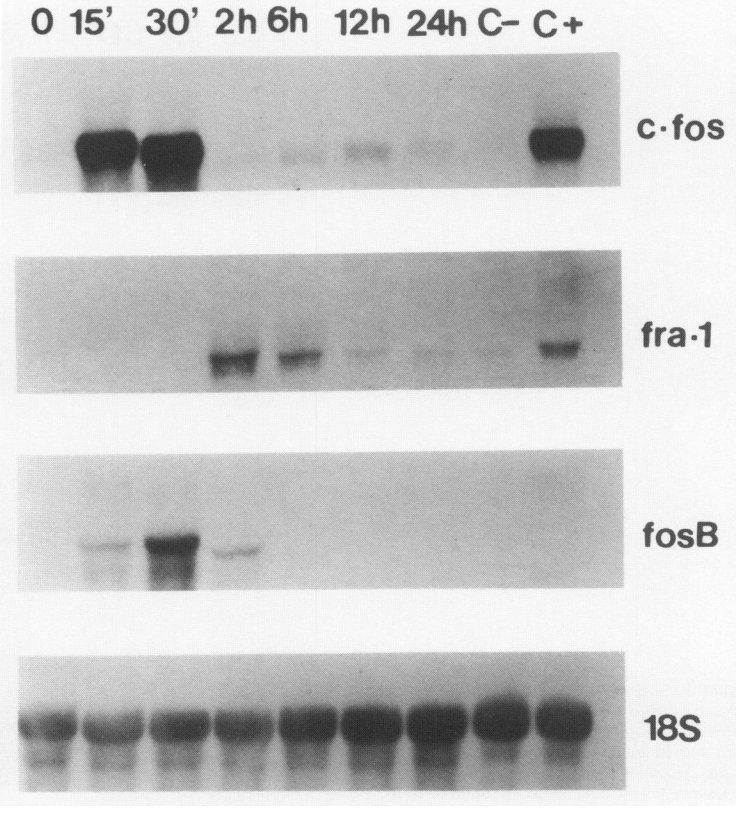

Figure 1. *A representative series of Northern blots hybridized to the indicated probes. Shown are the steady-state levels of* fos *members at various times after BDE. Total whole aortic tissue RNA (15 μg/lane) was processed for Northern blotting as described[7] and subjected to autoradiography. Note the biphasic increase in c-*fos *that is absent for other* fos *members and the delayed onset of* fra-*1. Control lanes represent osteosarcoma (U2OS) cells in the absence (C−) or presence (C+) of 10 μg/ml cycloheximide. Exposure times are as follows: c-*fos, *24 hours;* fra-*1, 5.5 days;* fosB, *38 hours. Equal RNA loading is demonstrated by hybridizing the blot to an 18S rRNA probe.*

ure 2). Like c-*fos*, levels of c-*jun* mRNA increased 20-fold between 15 and 30 minutes after BDE. Transcripts of c-*jun* decreased by 2 hours and remained at similar levels up to 24 hours (Figure 2). The expression of *junB* was weak, but easily demonstrated between 15 and 30 minutes, after which transcripts diminished to undetectable levels. Although *junD* is notably expressed in non-BDE whole aortic tissue, a fourfold increase in steady-state mRNA was observed 30 minutes after vascular injury. This increase was transient as *junD* transcripts returned to basal levels by 6 hours. Increases in *fos* and *jun* family members were not due to a universal increase in mRNA expression since transcripts of c-*fms*, c-*myb*, c-*Ha-ras*, and β-actin showed few or no changes over the time course studied (Figure 3).

Fos *and* Jun *Immunoreactivity in the Rat Aorta after BDE*

To determine whether *fos* and *jun* mRNAs are translated into immunoreactive proteins and to elucidate the spatial distribution of such products, rat aortic sections were examined by immunocytochemistry 2 hours after BDE. This time point was studied because Fos and Jun proteins are readily observed 2 hours after stimulation of cells in culture and *in vivo*.[18,26] At least 10 sections from the thoracic aorta of three rats were examined independently by three observers.

Non-BDE vessels showed no detectable Fos immunoreactivity (Figure 4A). In contrast, Jun products were faintly detected in SMC nuclei throughout non-BDE vessels (Figure 4B). Such Jun protein expression is consistent with the prominent *junD* signal observed in these vessels. Two hours after BDE, notable increases in Fos and Jun immunoreactive products were observed (Figure 4, C and D, respectively). Interestingly, both Fos and Jun staining were prevalent in SMC nuclei closest to the lumen, and Jun staining was consistently greater than Fos. Although most Fos and Jun staining appeared concentrated in SMC nuclei close to the lumen, some positive staining was observed at all levels of the aorta including cells of the adventitia (data not shown). The latter result exemplifies the importance of isolating medial SMC when studying SMC gene expression *in vivo*.[7,24]

Substituting Fos or Jun antibodies with normal rabbit IgG revealed no immunoperoxidase staining

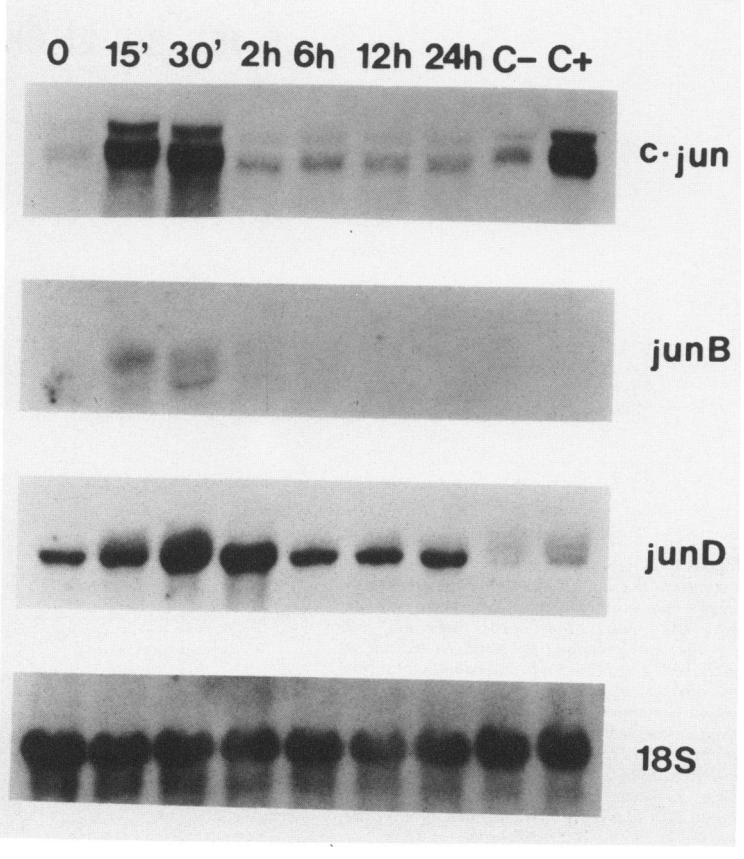

Figure 2. *mRNA expression of* jun *family members at various times after BDE. Note the obvious expression of* junD *in non-BDE (time 0) vessels and, to a lesser extent, c-*jun. *The control lanes are as described in the legend to Figure 1. Exposure times are as follows: c-*jun, *21 hours;* junB, *38 hours; and* junD, *38 hours.*

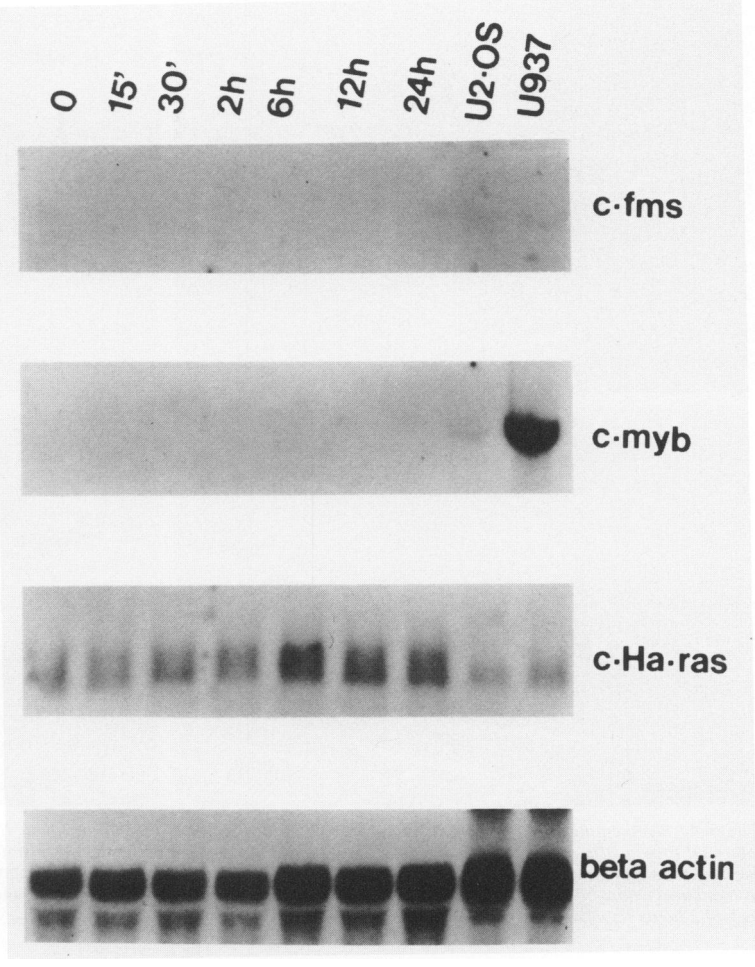

Figure 3. *This figure demonstrates that increases in IEG expression are not due to a generalized increase in mRNA expression in whole aortic tissue. Exposure times are as follows: c-fms, 6 days; c-myb, 7 days; c-Ha-ras, 2 days; and β-actin, 18 hours.*

in 2-hour BDE aortae (Figure 5A). As an additional control, some sections were incubated with an antibody that recognizes the core antigen of hepatitis B. No staining was observed with the latter antibody (data not shown).

To ascertain whether CHX could abolish Fos and Jun immunoreactive staining, rats underwent BDE and were immediately injected with a low dose of CHX. Such a dose of CHX results in an 80% inhibition in SMC protein synthesis.[17] The results in Figure 5B show that Jun immunoreactivity was markedly suppressed 2 hours after combined BDE/CHX treatment. Similar inhibition was noted for Fos staining (data not shown). These results provide further evidence for the specificity of the antisera and demonstrate the need for new protein synthesis for Fos and Jun immunoreactive detection after BDE.

Discussion

Immediate early genes, particularly those encoding transcription factors, are among the first genes induced in response to a variety of extracellular cues.[9,12,27–29] Such rapid IEG expression implies that their products subserve important cellular functions early following stimulation. Depending on the context, these functions may relate to proliferation, differentiation or some other activity. For example, IEG induction is observed during kidney and liver regeneration,[10,11] cornification of keratinocytes,[19] and brain seizures.[18] Understanding the function of IEG products in these experimental settings is vital because cell culture studies indicate that immediate early proteins play a pivotal role in cellular phenotypic change.[13,30,31]

We have combined Northern blotting and immunocytochemistry to demonstrate rapid mRNA expression of *fos* and *jun* family members as well as Fos and Jun immunoreactive products in the rat aorta after BDE. Steady-state levels of c-*fos*, *fosB*, c-*jun*, *junB*, and *junD* are elevated severalfold within 15 minutes of BDE. The rapid onset of such mRNA induction coincides with permeation of platelet factors into the vessel wall,[1] suggesting that platelet-derived factors activate SMC gene expression early

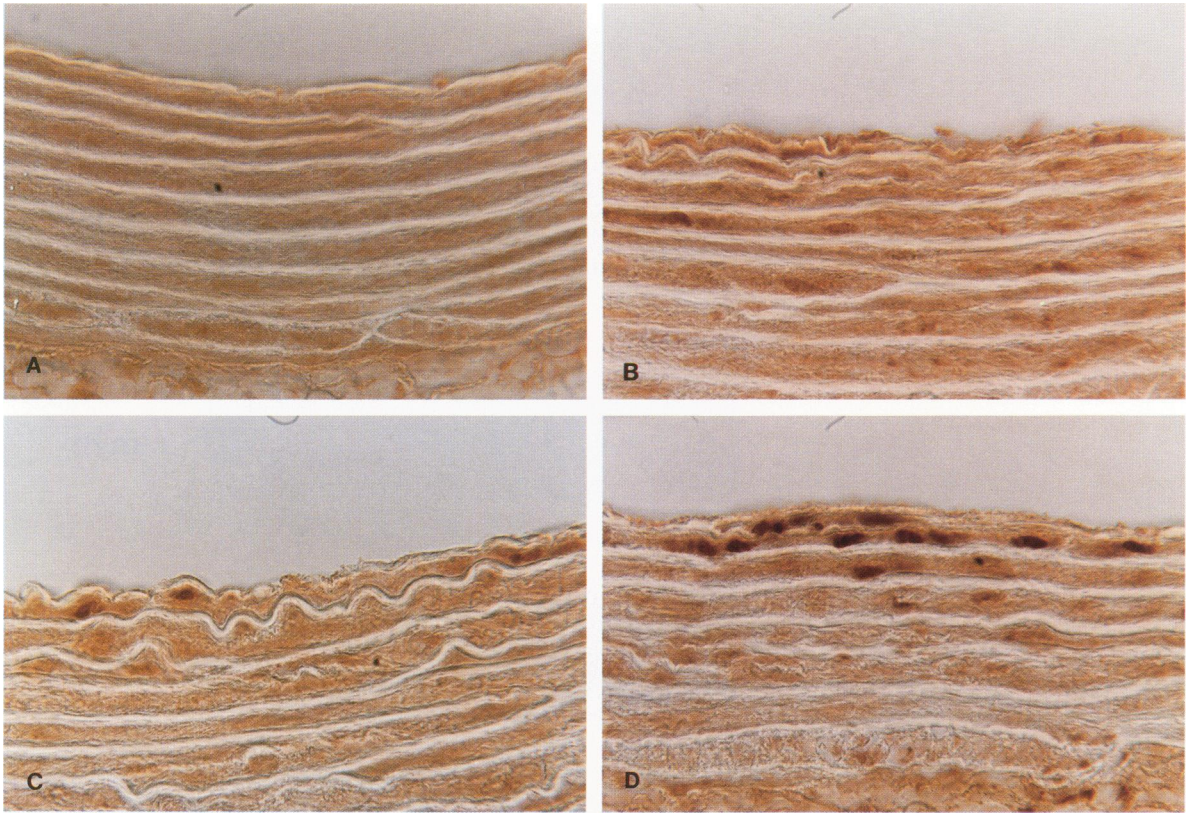

Figure 4. *Representative sections taken from non-BDE aortae* **(panels A** *and* **B)** *or 2-hour BDE aortae* **(panels C** *and* **D).** *No immunostaining is observed in non-BDE aortae incubated with a Fos antibody* **(panel A),** *whereas light Jun staining is detected in such vessels* **(panel B).** *BDE elicits strong Jun immunostaining that predominates at the luminal surface 2 hours after injury* **(panel D).** *A similar pattern is observed when Fos antisera is incubated with 2-hour BDE sections, albeit the staining is less intense* **(panel C).** *The latter may be due to the weaker* Fos *mRNA expression observed before 2 hours (compare Figures 1 and 2). Final magnification is* ×630.

after vascular injury. Indeed, levels of IEG transcripts diminish markedly when BDE is performed in the absence of blood (data not shown). Studies are in progress to ascertain whether serum and/or plasma factors mediate SMC IEG induction following vascular injury.

Immunocytochemistry data reveal some Jun staining in non-BDE aortic SMC. This basal Jun expression suggests that Jun-Jun homodimers are present under normal conditions in rat aortic SMC. Such expression may reflect JunD immunoreactivity because *junD* transcripts are readily observed in non-BDE vessels (see Figure 2). This possibility can be tested by utilizing antibodies that react specifically with each Jun protein.[13]

Two hours after BDE, Fos and Jun proteins are elevated, indicating that their respective mRNA transcripts are translated into immunoreactive products. Translation of *fos* and *jun* mRNA molecules is further substantiated by the absence of Fos and Jun staining when animals undergo combined BDE and CHX treatment. Cycloheximide attenuates SMC growth factor gene induction after BDE and immediate early proteins may be requisite for such gene activation.[17] The results reported here further corroborate a link between IEG products and SMC growth factor gene induction. We speculate that IEG products encoding transcription factors, such as Fos and Jun, act as early mediators of SMC autocrine growth by stimulating growth factor gene expression.

The greater Jun immunoreactivity observed in SMC nuclei 2 hours after BDE may be due to a greater affinity of the Jun antisera for its antigen. Alternatively, greater Jun protein expression may relate to the absence of certain *fos* mRNA molecules (ie, *fra*-1) as compared with *jun* before 2 hours after BDE (compare Figures 1 and 2). Again, specific antibodies that recognize each Fos and Jun member will clarify their relative expression. Because *fra*-1 mRNA levels show a delayed onset of expression as compared to other *fos* and *jun* members, a more detailed time course study may uncover further increases in Fos immunoreactivity at later time points.

Not all SMC of the media express Fos and Jun proteins 2 hours after BDE. Thus, not all SMC may be activated after BDE. This notion is consistent with

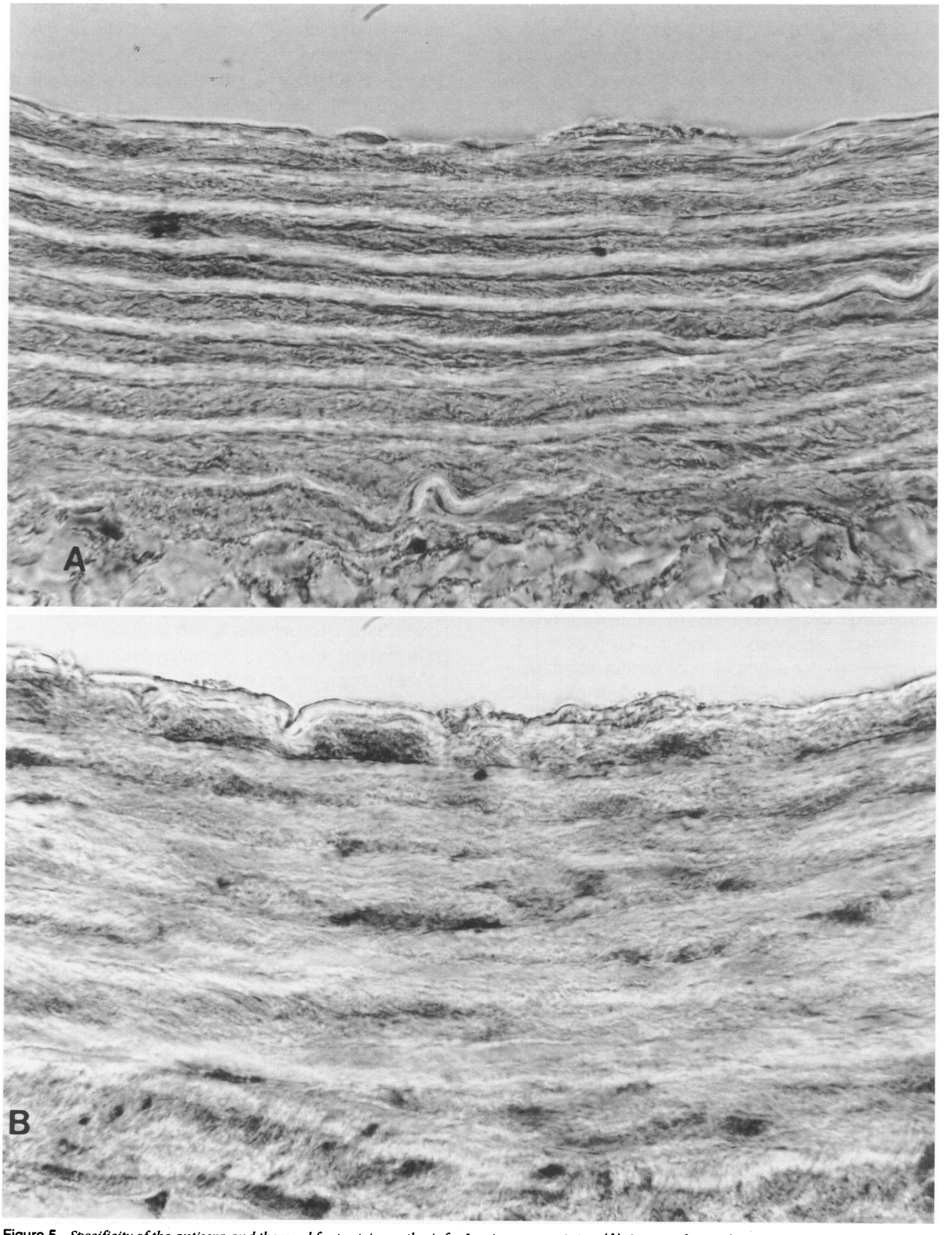

Figure 5. *Specificity of the antisera and the need for protein synthesis for Jun immunostaining.* **(A)** *Sections from 2-hour BDE aortae were incubated with rabbit IgG (1:2000) for 7 days and processed exactly as the sections exposed to Fos and Jun antisera.* **(B)** *Animals undergoing BDE received 7.5 mg/kg cycloheximide, and aortic tissue was isolated 2 hours later for immunoperoxidase staining with the Jun antisera. Prints taken with Kodak TMAX-100 black and white film. Final magnifications are ×630.*

data showing only 30 to 40% of medial SMC undergoing DNA synthesis in response to vascular balloon injury.[3] The expression of Fos and Jun proteins in a subpopulation of medial SMC following BDE may relate to the degree of SMC phenotypic modulation and/or their responsiveness to growth-promoting stimuli. Combining Fos and Jun staining with tritiated thymidine autoradiography will clarify whether the cells expressing these molecules represent SMC destined to undergo DNA synthesis.

Both Fos and Jun antibodies show staining predominantly in SMC nuclei at the luminal border. The focal concentration of Fos and Jun proteins in this region raises interesting speculation pertaining to the function of these transcription factors. Burns and colleagues[32] demonstrated a gradient of tritiated thymidine uptake across the vessel wall after BDE with luminal SMC incorporating more tritiated thymidine than SMC situated deeper in the media. Inasmuch as Fos and Jun are implicated in DNA replication,[33,34] the gradient of thymidine uptake across the vessel wall may be due to the higher expression of Fos and Jun at the luminal border. The presence of several *fos* and *jun* mRNA molecules at 24 hours suggests that their proteins may be available to participate in DNA synthesis. This hypothesis will require experiments specifically knocking out Fos or Jun molecules by, for example, homologous recombination and measuring DNA specific activity after BDE.

In addition to DNA synthesis, SMC undergo migration toward the intima of the injured vessel.[4] Platelet-derived material is postulated to play an important role in this process.[35–37] Because platelet-derived growth factors stimulate Fos and Jun expression,[26,38] SMC migration may occur, in part, through the activation of these immediate early transcription factors by released platelet material.[1] It is also possible that in response to the vascular injury, intravascular release of growth factors stimulates Fos and Jun expression. The appearance of functionally active Fos-Jun dimers in medial SMC could stimulate genes encoding proteins necessary for migration. For example, several proteases that may participate in this process contain AP-1 recognition sequences in their 5′ promoter/enhancer region and are transactivated by Fos-Jun dimers. Such genes include urokinase plasminogen activator,[39] transin,[40] and collagenase.[41] Thus, platelet/blood-derived (or intravascularly released) material may stimulate Fos and Jun expression in luminal SMC, leading to functional dimers that could direct transcription of proteases involved in the movement of SMC from the media to the intima during arterial remodelling.

At least one growth factor, transforming growth factor-β1 (TGF-β1), has AP-1–responsive elements in its 5′ region.[15] In this regard, TGF-β1 mRNA shows delayed induction with respect to *fos* and *jun* members, and treatment with CHX attenuates its expression.[17] Moreover, CHX treatment abolishes Fos and Jun immunoreactivity in SMC after BDE, indicating that active translation is necessary for their appearance in SMC nuclei. Taken together, the data reported here are consistent with the notion that immediate early proteins, such as Fos and Jun, stimulate autocrine growth factor gene expression in the vessel wall. Studies combining *in situ* hybridization of TGF-β1 with immunocytochemistry of Fos and Jun will strengthen this thesis.

In summary, acute balloon injury to the rat aorta elicits sequential mRNA and protein expression of two families of immediate early genes. The induction of several *fos* and *jun* transcripts within 15 minutes of BDE represents the earliest molecular markers of *in vivo* SMC activation, and the presence of Fos and Jun proteins in SMC nuclei suggests that they function as early mediators of SMC phenotypic change. The luminal concentration of Fos and Jun proteins coincides with the permeation of platelet factors into the vessel wall and SMC thymidine incorporation.[1,32] Such protein expression may contribute to the initiation of SMC DNA synthesis and migration. We propose that Fos and Jun protein expression be used as an early assay for an agonist's or antagonist's efficacy in modulating SMC growth/migration *in vivo*. Finally, the luminal pattern of Fos and Jun expression may be exploited in the design of treatment protocols to suppress their activity with, for example, transfected antisense constructs.

Note added in proof

After submission of this paper, Hsu et al (Mol Cell Biol 1992, 12:4654–4665) demonstrated Fos and Jun protein induction in regenerating liver by Western blotting.

Acknowledgments

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References

1. Goldberg ID, Stemerman MB, Handin RI: Vascular permeation of platelet factor four following endothelial injury. Science 1980, 209:611–612
2. Goldberg ID, Stemerman MB, Schnipper LE, Ransil BJ, Crooks GW, Fuhro RL: Vascular smooth muscle cell kinetics: A new assay for studying patterns of cellular proliferation in vivo. Science 1979, 205:920–921
3. Clowes AW, Reidy MA, Clowes MM: Kinetics of cellular proliferation after arterial injury. I. Smooth muscle growth in the absence of endothelium. Lab Invest 1983, 49:327–333
4. Clowes AW, Schwartz SM: Significance of quiescent smooth muscle migration in the injured rat carotid artery. Circ Res 1985, 56:139–145
5. Liu MW, Roubin GS, King III SB: Restenosis after coronary angioplasty. Potential biologic determinants and role of intimal hyperplasia. Circulation 1989, 79:1374–1387
6. Adams PC, Lam JY, Badimon L, Chesebro JH, Fuster V: Interactions of platelets and vessel wall in the development of restenosis after coronary angioplasty. Ann N Y Acad Sci 1989, 516:605–620
7. Miano JM, Tota RR, Vlasic N, Danishefsky KJ, Stemerman MB: Early proto-oncogene expression in rat aortic smooth muscle cells following endothelial removal. Am J Pathol 1990, 137:761–765
8. Majesky MW, Reidy MA, Bowen-Pope DF, Hart CE, Wilcox JN, Schwartz SM: PDGF ligand and receptor gene expression during repair of arterial injury. J Cell Biol 1990, 111:2149–2158
9. Bravo R: Growth factor-responsive genes in fibroblasts. Cell Growth Different 1990, 1:305–309
10. Cowley BD, Jr, Chadwick LJ, Grantham JJ, Calvet JP: Sequential proto-oncogene expression in regenerating kidney following acute renal injury. J Biol Chem 1989, 264:8389–8393
11. Mohn KL, Laz TM, Hsu JC, Melby AE, Bravo R, Taub R: The immediate-early growth response in regenerating liver and insulin-stimulated H-35 cells: Comparison with serum-stimulated 3T3 cells and identification of 41 novel immediate-early genes. Mol Cell Biol 1991, 11:381–390
12. Vogt PK, Bos TJ: jun: Oncogene and transcription factor. Adv Cancer Res 1990, 55:1–35
13. Kovary K, Bravo R: The Jun and Fos protein families are both required for cell cycle progression in fibroblast. Mol Cell Biol 1991, 11:4466–4472
14. Curran T, Franza BR, Jr: Fos and Jun: The AP-1 connection. Minireview. Cell 1988, 55:395–397
15. Kim SJ, Angel P, Lafyatis R, Hattori K, Kim KY, Sporn MB, Karin M, Roberts AB: Autoinduction of transforming growth factor beta1 is mediated by the AP-1 complex. Mol Cell Biol 1990, 10:1492–1497
16. Majesky MW, Lindner V, Twardzik DR, Schwartz SM, Reidy MA: Production of transforming growth factor beta one during repair of arterial injury. J Clin Invest 1991, 88:904–910
17. Miano JM, Vlasic N, Tota RR, Stemerman MB: Smooth muscle cell immediate-early gene and growth factor activation follows vascular injury: a putative *in vivo* mechanism for autocrine growth. Arterio Thromb 1993, 13:211–219
18. Morgan JJ, Cohen DR, Hempstead JL, Curran T: Mapping patterns of c-fos expression in the central nervous system after seizure. Science 1987, 237:192–197
19. Fisher C, Byers MR, Iadarola MJ, Powers EA: Patterns of epithelial expression of Fos protein suggest important role in the transition from viable to cornified cell during keratinization. Development 1992, 111:253–258
20. Yang G, Koistinaho J, Zhu S, Hervonen A: Light and electron microscopic evidences of the presence of c-fox-like immunoreactivity in the rat adrenal cortex. Histochemistry 1989, 93:217–221
21. Kawakita N, Seki S, Sakaguchi H, Yanai A, Kuroki T, Mizoguchi Y, Kobayashi K, Monna T: Induction of proto-oncogene c-jun product in rat liver after partial hepatectomy. Gastroenterol Jpn 1990, 25:512
22. Stemerman MB: Thrombogenesis of the rabbit arterial plaque; an electron microscopic study. Am J Pathol 1973, 73:7–18
23. Mugnaini E, Dahl AL: Zinc-aldehyde fixation for light-microscopic immunocytochemistry of nervous tissues. J Histochem Cytochem 1983, 31:1435–1438
24. Cassis LA, Lynch KR, Peach MJ: Localization of angiotensinogen messenger RNA in rat aorta. Circ Res 1988, 62:1259–1262
25. Ryseck RP, Bravo R: c-Jun, JUN B, JUN D differ in their binding affinities to AP-1 and CRE consensus sequences: Effect of FOS proteins. Oncogene 1991, 6:533–542
26. Muller R, Bravo R, Burckhardt J, Curran T: Induction of c-fos gene and protein by growth factors precedes activation of c-myc. Nature 1984, 312:716–720
27. Morgan JI, Curran T: Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes fos and jun. Annu Rev Neurosci 1991, 14:421–451
28. Herschman HR: Primary response genes induced by growth factors and tumor promoters. Annu Rev Biochem 1991, 60:281–319
29. Nathans D, Lau LF, Christy B, Hartzell S, Nakabeppu Y, Ryder K: Genomic response to growth factors. Cold Spring Harbor Symp Quant Biol 1988, 53:893–900
30. Okuno H, Suzuki T, Yoshida T, Hashimoto Y, Curran T, Iba H: Inhibition of jun transformation by a mutated fos gene: design of an anti-oncogene. Oncogene 1991, 6:1491–1497
31. Lachman HM, Skoultchi AI: Expression of c-myc changes during differentiation of mouse erythroleukaemia cells. Nature 1984, 310:592–594
32. Burns ER, Spaet TH, Stemerman MB: Response of the arterial wall to endothelial removal: an autoradiographic study. Proc Soc Exp Biol Med 1979, 159:473–477

33. Murakami Y, Satake M, Yamaguchi-Iwai Y, Sakai M, Muramatsu M, Ito Y: The nuclear protooncogenes c-jun and c-fos as regulators of DNA replication. Proc Natl Acad Sci USA 1991, 88:3947–3951
34. Scanlon KJ, Jiao L, Funato T, Wang W, Tone T, Rossi JJ, Kashani-Sabet M: Ribozyme-mediated cleavage of c-fos mRNA reduces gene expression of DNA synthesis enzymes and metallothionein. Proc Natl Acad Sci USA 1991, 88:10591–10595
35. Friedman RJ, Stemerman MB, Wenz Ban, M.B., Moore S, Gauldie J, Gent M, Tiell ML, Spaet TH: The effect of thrombocytopenia on experimental arteriosclerotic lesion formation in rabbits. J Clin Invest 1977, 60:1191–1201
36. Fingerle J, Johnson R, Clowes AW, Majesky MW, Reidy MA: Role of platelets in smooth muscle cell proliferation and migration after vascular injury in rat carotid artery. Proc Natl Acad Sci USA 1989, 86:8412–8416
37. Ferns GAA, Raines EW, Sprugel KH, Motani AS, Reidy MA, Ross R: Inhibition of neointimal smooth muscle accumulation after angioplasty by an antibody to PDGF. Science 1991, 253:1129–1132
38. Pertovaara L, Sistonen L, Bos TJ, Vogt PK, Keski-Oja J, Alitalo K: Enhanced jun gene expression is an early genomic response to transforming growth factor beta stimulation. Mol Cell Biol 1989, 9:1255–1262
39. Nerlov C, Pergola F, de Cesare D, Caracciolo A, Blasi F, Johnsen M, Verde P: AP-1 and PEA3 binding elements in the urokinase enhancer mediate regulation by TPA, cAMP and glucocorticoids in HepG2 cells. J Cell Biochem 1992, 16:104
40. Kerr LD, Holt JT, Matrisian LM: Growth factors regulate transin gene expression by c-fos-dependent and c-fos-independent pathways. Science 1988, 242:1424–1427
41. Schonthal A, Herrlich P, Rahmsdorf HJ, Ponta H: Requirement for fos gene expression in the transcriptional activation of collagenase by other oncogenes and phorbol esters. Cell 1988, 54:325–334