Atherosclerotic plaques preferentially localize to areas of the vasculature with complex laminar or oscillatory blood flow. Prior data implicate matrix metalloproteinases (MMPs) in the initiation and progression of atherosclerotic lesions. In cultured endothelial cells, oscillatory but not unidirectional shear significantly increases MMP-9 mRNA as well as secretion of the MMP-9 protein (p < 0.05). In contrast, cell-associated protein levels of Tissue Inhibitor of MMP 1 (TIMP-1), an inhibitor of MMP-9, are insensitive to the shear regimen. To investigate transcriptional regulation of MMP-9 gene expression, we utilized retroviral-based reporter constructs containing different lengths of the human MMP-9 promoter. The activity of the full MMP-9 promoter is 3-fold higher (p < 0.05) in unidirectional shear compared with static conditions, and the activity is further increased ~10-fold by oscillatory shear (p < 0.01) over unidirectional flow. Our data identify a shear-sensitive binding site at −152 in the MMP-9 promoter. We show that the c-Myc transcription factor binds specifically to this site and that reporter constructs in which the c-Myc binding site was abolished lacked the shear responsiveness of native MMP-9 reporter constructs. Our results suggest that endothelial MMP-9 expression is flow-sensitive and is up-regulated by oscillatory flow via activation of c-Myc. This effect may contribute to the development and progression of atherosclerotic lesions in areas of vasculature that are subject to disturbed flow.

Remodeling of the extracellular matrix during the development and progression of atherosclerotic lesions depends upon matrix metalloproteinases (MMPs), and especially the gelatinase MMP-9 (1–4). This MMP is not produced under basal conditions by vascular cells (5, 6) and it is not detectable in normal human smooth muscle cells, healthy human arteries, and normal animal arteries (2, 7). However, MMP-9 is induced in vascular cells by cytokine stimulation (6, 8) and is constitutively secreted by inflammatory cells (5).

Atherosclerotic plaques preferentially form in specific areas of the vasculature, including the infrarenal aorta, the coronary arteries, and at the carotid bifurcation (9). Modeling and direct measurement techniques have revealed that these regions are areas of complex non-laminar blood flow patterns (10). Other work correlates intimal hyperplasia with areas of either low or oscillatory shear stresses (11, 12). Observations in both human and animal models have shown that arteries remodel to maintain a wall shear stress of around 15 dynes/cm² and that an inability to remodel hastens lesion progression (10). Despite this clear observation, the vascular cellular responses to physiologic mechanical stimuli (e.g. shear stress from blood flow and mechanical strain from blood pressure) remain less understood than the responses to biochemical stimuli.

This study examines the hypothesis that the pattern of flow regulates expression of MMP-9 in endothelial cells. Previous studies have demonstrated shear-sensitive expression of several genes in endothelial cells, including intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial nitric oxide synthase (eNOS) (13–15). However, the majority of previous investigations made comparisons between static cell culture and unidirectional flow systems and, thus, did not address the actual physiological conditions implicated in atherosclerosis, namely differences in the cellular response to steady unidirectional or disturbed flow. Intriguingly, when bidirectional (also referred to as oscillatory or disturbed) flow effects were investigated in vitro, endothelial cells were found capable of uniquely responding to the different environments (16–19).

The present study investigates the hypothesis that the expression of MMP-9 by endothelial cells is transcriptionally regulated in response to shear stress. To isolate the directional effects of shear, we have used a system in which the peak magnitude of shear stress is identical under unidirectional and bidirectional regimens, with only the direction changing. MMP-9 expression in this system was examined at the levels of both promoter activity and secreted protein. Specific promoter constructs were then utilized to investigate the mechanism of hemodynamic regulation of MMP-9 expression in endothelial cells.

MATERIALS AND METHODS

Cell Culture and Reagents—The murine lymphoid endothelial cell (MLEC) line (CRL-2167) was purchased from the American Type Culture Collection. Cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% calf serum. Primary human umbilical vein endothelial cells (HUVECs) were obtained from the Emory University Skin Disease Research Center, maintained in EGM-2MV medium (Clonetics), and used between passages 3 and 8. Human
umbilical vein endothelial cells were quiesced in M199 supplemented with insulin, transferrin, and selenium (ITS reagent;Cambrex). Retroviral Reporter Plasmids—The MMP-9 promoter was isolated from total human genomic DNA using the polymerase chain reaction. Oligonucleotide primers were used to introduce a BamHI and a HindIII site at the 5’ and 3’ respective ends of the amplified region, allowing cloning into the pcDNA3 retroviral vector immediately proximal to the luciferase coding sequence. The pcDNA3 plasmid is a modification of the pk8.2 retroviral plasmid (20) and contains on the opposite strand of the luciferase coding sequence a chloramphenicol acetyl transferase (CAT) coding sequence constitutively driven by the viral 5’-long terminal repeat promoter (Fig. 1). This construct allows for the simultaneous measurement of both the luciferase and CAT levels. The level of CAT is used as an internal control to normalize for variations in viral integration efficiency, copy number, and effects relating to chromosomal integration location. The pMyc-CC plasmid was created by mutating the c-Myc consensus binding domain TCAATG to TCCGGC using site direction mutagenesis (QuikChange XL, Stratagene). All constructs were sequenced and verified at the Emory University Microchemical Sequencing Facility.

Retroviral Production and Infection—Retroviruses were prepared by transient transfection of Phoenix helper virus-free amphotropic producer cells (21). To produce infectious retroviral supernatants, the cells at 50–80% confluence in 10-cm diameter dishes were transfected with the retroviral plasmids by using calcium phosphate and 25 μg chloroquine for 6–12 h before re-feeding with 25 ml of growth medium (GM). Twenty-four hours after initiating the transfection, the GM was aspirated and replaced with 9 ml of fresh GM before placing the dishes in a humidified 5% CO2 atmosphere at 32°C. The supernatant containing retroviral particles was harvested after 24 h and twice more at 12–18 h intervals thereafter, replenishing with 9 ml of GM each time. Each collected supernatant (9 ml) was filtered through a sterile 0.45-μm syringe tip cellulose acetate disk, aliquoted, snap-frozen in liquid nitrogen, and stored at −80°C. MLECs (~33% confluent) grown on 35-mm multiwell plates were infected with retroviruses by adding a thawed retroviral supernatant (2 ml) containing 8 μg/ml Polybrene to each well and spinning the cells at 2,500 rpm for 30 min at 32°C in a Beckman model GS-6R refrigerated centrifuge in a swinging bucket rotor before placing them in a 5% CO2 incubator at 37°C. This infection protocol was repeated twice more at 8–12 h intervals. Infection efficiency was assessed by using a LacZ reporter virus with subsequent colorimetric detection of infected cells using the 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) substrate and was found to be 90–95%.  

Electrophoretic Mobility Shift Assay (EMSA)—Nuclear extracts were prepared following the methods of Dignam et al. (23). Protein-DNA complexes were detected using biotin end-labeled double-stranded DNA probes prepared by annealing complementary oligonucleotides. Oligonucleotides were purchased from Operon Technologies and end-labeled with γ-[32P]ATP using terminal deoxynucleotidyl transferase (Promega) and biotin-14-dCTP (Invitrogen). The binding reaction was performed using the LightShift kit (Pierce). Briefly, nuclear extracts (4 μg of protein) and binding buffer were incubated on ice for 5 min in a volume of 20 μl, then the labeled probe (20 fmol) was added, and the reaction was allowed to incubate for an additional 25 min. For c-Myc supershift analysis, an anti-c-Myc monoclonal antibody (NeoMarkers, Ab-5; 1,300) or anti-human Max polyclonal antibody (Novus Biologicals; 1,300) secondary antibodies were horseradish peroxidase conjugated, and detection was performed using the ECL chemiluminescence kit (Amersham Biosciences).

Luciferase Assay for Promoter Activity—After exposure to shear, the infected MLECs were washed with phosphate-buffered saline and lysed in 1 ml of lysis buffer per 100-mm dish (62.5 mM Tris base, 4 mM EGTA, 1 mM glycerol, 1% Triton X-100, and 2 mM dithiothreitol, pH 7.8). After 10 min of incubation at room temperature, the lysates were collected and equal amounts were subjected to gelatin zymography. Gelatinolytic bands were quantified using Multi-Analyst densitometry software (Bio-Rad). The significance of differences in optical density of bands was analyzed by analysis of variance (ANOVA) and differences between groups were tested with a Student’s t test.
Oscillatory shear stress increases MMP-9 protein levels secreted by MLEC.èrection of MMP-9 into the cell culture medium following 6 h of exposure to shear stress (15 dynes/cm² for unidirectional and ±15 dynes/cm² at 1Hz for oscillatory) was measured by SDS-PAGE gelatin zymography. Oscillatory shear stress was found to increase secreted protein levels 2.7-fold over unidirectional shear (p < 0.05). Levels of TIMP-1, a specific inhibitor of MMP-9, were detected by Western blotting. Arb, arbitrary; Uni, unidirectional; Osc, oscillatory; Pro, proenzyme; Stat, static; +ve, positive control.

RESULTS

MMP-9 Expression Is Regulated by Hemodynamics—To study the secretion of both the latent enzyme and the proteolytically active enzyme, levels of MMP-9 were measured by gelatin SDS-PAGE zymography (Fig. 2). An ~3-fold higher level of total (inactive plus active) secreted enzyme is observed from MLEC subjected to a 6-hour regimen of bidirectional shear compared with the response in cells that remained under unidirectional flow (p < 0.05). MLEC expression of MMP-9 following unidirectional flow appears to be lower than that of cells under static culture conditions, but the differences were not significant (p = 0.15). Physiologically, MMP activity is known to be restricted additionally by the presence of TIMPs. To verify that the increased MMP-9 levels were not accompanied by a corresponding increase in TIMP-1, a specific inhibitor of MMP-9, TIMP-1 protein levels were measured by Western blotting. Neither secreted nor cell-associated TIMP-1 levels were observed to be changed by either unidirectional or bidirectional flow.

Messenger RNA levels in MLEC subject to unidirectional or bidirectional shear stress were measured using quantitative real time reverse transcription PCR (Fig. 3). Compared with levels in static conditions, the MMP-9 message was unchanged following either 3 or 6 h of exposure to unidirectional shear. However, after exposure of MLEC to oscillatory shear for 3 h, MMP-9 mRNA levels increased 3-fold, and after 6 h the levels had increased 8-fold (*, p < 0.05 for each versus static control).

MMP-9 m RNA levels increased 3-fold, and after 6 h levels increased 8-fold (p < 0.05 for each versus static control).

MMP-9 promoter activity was investigated by using a series of retroviral constructs in which varying lengths of the human MMP-9 promoter were used to drive expression of a luciferase reporter gene (see Fig. 1). To normalize for variation in viral infection efficiency and integration copy number, the retroviral construct also contained a chloramphenicol acetyl transferase gene constitutively driven by the viral 5′-long terminal repeat. The specific MMP-9 promoter fragments tested for activity included 2,200, 670, 498, 153, 93, and 73 bp of sequence upstream of the transcription start site (Fig. 4).

Using 2.2 kb of the promoter region, a 6-h regimen of unidirectional shear increased MMP-9 promoter activity 3-fold over the static control (p < 0.05). Additionally, 6 h of bidirectional shear resulted in an 8-fold further increase, representing a net 25-fold increase over the static control (Fig. 4, p < 0.01). Stepwise truncation was used to reveal areas of the MMP-9 promoter that are necessary for this hemodynamic responsiveness. Promoter constructs containing at least the proximal 153 bp were regulated by the hemodynamic shear regimen. However, this response was absent in further promoter deletions.

Characterization of the Shear-sensitive Transcription Factor Binding Element—The 60-bp region between −153 and −93 of the MMP-9 promoter was further dissected in detail for shear-dependent transcriptional regulation utilizing the MatInspector sequence analysis tool (core similarity > 0.75; matrix similarity > 0.75) to reveal putative transcription factor binding sites. In addition to a 42-base pair CA microsatellite repeat element, several potential transcription factor binding sites adjacent to the repeat were identified. As a first approach to determine whether any of these putative sites were active, EMSA analysis was performed to reveal protein binding to this shear-sensitive promoter region.

Oligonucleotides corresponding to the microsatellite repeat, the adjacent region (−133 to −155) and a portion of the promoter external to the shear-sensitive region (−154 to −176), were investigated. The nuclear extracts were derived from cells subject to 3 h of bidirectional flow. A prominent shifted complex was detected with probes consisting of DNA immediately adjacent to the microsatellite repeat (−133 to −155) but was not present when using the microsatellite repeat itself or the probe external to this putative shear-sensitive region (Fig. 5A).

The 23-base sequence in the shear-sensitive promoter region contained several putative transcription factor binding sites. To identify the specific element, we undertook EMSA analysis utilizing a series of probes in which 2-bp mutations had been introduced. This strategy revealed a 6-bp subregion that was necessary for transcription factor binding (Fig. 5B). Analysis of the sequence suggests that this region contains a putative c-Myc binding domain. The addition of an anti-c-Myc antibody to the EMSA binding results in a supershifted complex.
whereas the addition of an isotype control antibody has no effect (Fig. 5C). The formation of this complex is time-dependent and increases strongly over the initial 120 min of exposure to shear stress. The transcription factor c-Myc is believed to be active only after forming a heterodimeric complex with Max, a basic helix-loop-helix leucine zipper (bHLHZip) protein (24). We thus hypothesized that changes in either c-Myc nuclear abundance or Max nuclear abundance could be responsible for the shear stress-sensitive regulation of MMP-9 expression in endothelial cells. To investigate the nuclear protein levels, Western blots were run on nuclear protein extracts that had been prepared previously for EMSA analysis. We found that neither the levels of nuclear c-Myc nor Max were affected by exposure to 60 or 120 min of shear. Additionally, the gel supershift EMSA was repeated using an anti-Max antibody. However, instead of causing a supershift, addition of the antibody disrupted the complex formation (Fig. 5C).

**FIG. 5. c-Myc binds specifically to the MMP-9 promoter after exposure to oscillatory shear stress.** A. EMSAs show that protein binding only occurs in a region of the promoter sensitive to shear (region B). DNA-protein interaction was not observed in the microsatellite repeat (region dCA) or in an adjacent promoter region (region A). B. EMSA showed that the DNA-protein interaction is localized to the 5′-end of the shear-responsive region. DNA probes with 2-bp mutations (B1, B2, etc.) were used to identify the specific portion of the MMP-9 promoter to which the shear-responsive factor binds. The putative c-Myc binding element is boxed. WT, wild type; neg, negative; pos, positive. C. EMSAs showing the rapid induction of a DNA-binding factor in endothelial cells subject to oscillatory shear stress. Gel supershift with the addition of an anti-c-Myc antibody (cMyc Ab) but not with an isotype control (IgG1) identifies c-Myc as the shear sensitive transcription factor in the MMP-9 promoter. Addition of an anti-Max antibody (Max Ab) specifically abolished the complex formation.

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**FIG. 6. c-Myc is involved specifically in the hemodynamic regulation of MMP-9 expression in endothelial cells.** MLECs were infected with reporter constructs in which the putative c-Myc binding domain was specifically mutated to disrupt the DNA-protein interaction. Following 6 h of exposure to shear stress (15 dynes/cm² for unidirectional and ±15 dynes/cm² at 1 Hz for oscillatory), promoter activity was determined using luciferase luminescence normalized by CAT activity. Abolition of the binding element eliminated the shear responsiveness of the MMP-9 promoter (*, p < 0.01). Uni, unidirectional; Osc, oscillatory.

**Oscillatory Shear Up-regulates MMP-9 in Endothelial Cells**
Oscillatory Shear Up-regulates MMP-9 in Endothelial Cells

transcription was measured by luciferase reporter assay from a promoter construct with a mutated c-Myc binding domain (CT-CATG → CTCGTT) in response to shear stress. The mutation was created in the context of a larger construct containing the 670-bp fragment of the promoter, a construct found previously to be shear-sensitive, and it was mutated specifically to abolish the c-Myc binding element. Abolition of this site conferred complete insensitivity to shear stress in MLEC (Fig. 6).

DISCUSSION

This study establishes that MMP-9 expression by endothelial cells is regulated in response to the hemodynamic environment. Furthermore, the data show that regulation discriminates between shear patterns and occurs at the level of transcription, and that a necessary element of MMP-9 promoter activation by shear is the binding of a constitutively expressed c-Myc:Max heterodimer to a non-canonical DNA element (CT-CATG versus E-box CACGTT). Because MMP-9 is implicated in vascular disease, this study offers further insight into the mechanistic relation between hemodynamics and atherosclerosis in those areas of the vasculature subject to complex flow patterns and/or low mean shear stresses.

In some of the initial studies of endothelial hemodynamic behavior, Hemlinger et al. found that the cells align in the direction of flow in response to culture under unidirectional flow, but not when cultured under bidirectional flow (25). More recently, groups have shown that bidirectional shear increases tissue factor expression, resulting in a pro-coagulant phenotype for endothelial cells in areas likely to have established atherosclerotic lesions (18). Other researchers have shown that, whereas endothelial nitric oxide synthase expression and activity is up-regulated by unidirectional shear stress (26), oscillatory shear stress reduces the levels of nitric oxide (19), a potent anti-atherogenic molecule, in those areas of the vasculature with complex hemodynamic flows. Oscillatory shear also increases endothelial NADH oxidase activity, resulting in increased intracellular superoxide concentrations (7).

Two of the key processes in atheroma development are leukocyte infiltration into the vessel wall (27) and smooth muscle cell migration into the developing neointima (28). Because both of these processes involve degradation of the intimal extracellular matrix and because neither process is broadly observed in healthy areas of the vasculature, we hypothesized that MMP expression in endothelial cells might be aberrantly regulated at sites in blood vessels where the endothelial cells are exposed to complex hemodynamic forces. An increase in MMP-9 expression by endothelial cells would cause a local increase in the enzyme concentration with potential subsequent proteolysis of the extracellular membrane. It is probable that this would lead to a decrease in endothelial cell viability and a loss of the endothelial barrier function.

This study utilized the MLEC endothelial cell line for the reporter constructs because of the high efficiency of retroviral infection and extended lifetime in culture. This is a transformed cell line and may respond differently than primary endothelial cells; thus, primary cells (human umbilical vein endothelial cells) were utilized in all gel shift assays to confirm our findings. Further confirming our data, we have observed that oscillatory shear increases MMP-9 secretion in primary cultures of murine endothelial cells. Interestingly, the increase in MMP-9 following bidirectional shear was observed as an increase in the form of the enzyme, not as a pro-enzyme. We interpret this finding as suggestive for conversion of increased levels of latent MMP-9 to the activated form, possibly due to the increased production of reactive oxygen species (ROS) shown to occur in endothelial cells subjected to disturbed shear stress environments (16). Our previous studies demonstrated that interaction with ROS leads to activation of latent MMP-2 and MMP-9 (29). Therefore, a common characteristic of MMP-9 expression in several endothelial cell phenotypes is its regulation in response to shear. In this study, unidirectional shear stress increased MMP-9 promoter activity as measured by the luciferase reporter, but no corresponding increase in mRNA or protein secretion was observed. We believe that this apparently paradoxical effect may be an acute response to the initiation of flow. The presence of a luciferase signal but not an increase in MMP-9 mRNA could be attributed to the longer lifetime of the luciferase protein. It is also possible that the stability of the MMP-9 message is regulated by the hemodynamic environment, a mechanism that was not investigated in this study but has been observed for the endothelial nitric oxide synthase gene (15). Furthermore, the luciferase reporters contain only a 2.2-kb portion of the full MMP-9 promoter, which may result in “leaky” expression not observed at the message level.

Prior work investigating the mechanisms of transcriptional regulation has identified several promoter elements important in MMP-9 gene regulation. Sato et al. showed that AP-1 is necessary but not sufficient for the induction of MMP-9 in an osteosarcoma-derived cell line and that cooperation with NF-κB or SP-1 is required for normal expression (30). Others have also implicated AP-1 in the transcriptional induction of MMP-9 by phorbol esters and tumor necrosis factor α (TNF-α) (31). Munaut et al. used a LacZ reporter system to investigate promoter elements in the MMP-9 gene in murine embryos and were able to show in vivo that specific sequence elements between the −2722 to −7745 upstream region were responsible for cell type-specific expression control in osteoclasts and migrating keratinocytes (32). AP-2 has also been shown to be capable of regulating MMP-9 activity levels in an in vivo wound-healing model (33). It appears that different pathways regulate MMP-9 expression depending on the cell type and environment. We found that shear-dependent regulation depends on c-Myc, and the overall conclusion drawn from the body of literature and the current study is that the body must maintain very tight control on the activity of metalloproteinases in order to maintain proper cellular, tissue, and organ function. When several factors conspire to increase the levels of MMP-9, pathologies such as metastatic cancer, arthritis, or atherosclerosis can result.

The roles of mechanical forces in vascular biology are still in the process of being dissected and understood at a molecular level. This report shows that the MMP-9 gene is not only shear-sensitive but that an oscillatory hemodynamic regimen exerts different effects on gene expression than does steady laminar flow. The data also show that this transcriptional regulation is due to the action of the c-Myc transcription factor acting through an element at −152 in the MMP-9 promoter. This finding is significant because it is one of the first studies to correlate changes in expression of a protein that has known roles in the development and progression of atherosclerosis with the hemodynamic conditions that predict said pathology.

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REFERENCES

1. Henney, A. M., Wakeley, P. R., Davies, M. J., Foster, K., Hembry, R., Murphy, G., and Humphries, S. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 8154–8158
2. Galis, Z. S., Sukhova, G. K., Lark, M. W., and Libby, P. (1994) J. Clin. Invest. 94, 2493–2503
3. Shah, P. K., Falk, E., Badimon, J. J., Fernandez-Ortiz, A., Mailhaux, A., Villareal-Levy, G., Fallon, J. T., Regenstorm, J., and Foster, V. (1995) Circulation 92, 1565–1569
4. Brown, D. L., Hibbs, M. S., Kearney, M., Loushine, C., and Isner, J. M. (1995) Circulation 91, 2125–2131
5. Hanemaaijer, R., Koolewijn, P., le Clercq, L., de Vree, W. J., and van Hinsbergh, V. W. (1995) Biochem. J. 326, 803–809
6. Galis, Z. S., Muszynski, M., Sukhova, G. K., Simon-Morrissey, E., Unemori,
E. N., Lark, M. W., Amenta, E., and Libby, P. (1994) Circ. Res. 75, 181–189
7. Galis, Z. S., Sukhova, G. K., Kranzhofer, R., Clark, S., and Libby, P. (1995) Proc. Natl. Acad. Sci. U. S. A 92, 402–406
8. Rao, V. H., Singh, R. K., Delimont, D. C., Finnell, R. H., Bridge, J. A., Neff, J. R., Garvin, B. P., Pickering, D. L., Sanger, W. G., Bucher, B. A., and Schaefer, G. B. (1999) Int. J. Oncol. 14, 291–300
9. Glagov, S., Zarins, C., Giddens, D. P., and Ku, D. N. (1988) Arch. Pathol. Lab. Med. 112, 1018–1031
10. Giddens, D. P., Zarins, C. K., and Glagov, S. (1993) J. Biomech. Eng. 115, 588–594
11. Ku, D. N., Giddens, D. P., Zarins, C. K., and Glagov, S. (1985) Arteriosclerosis 5, 293–302
12. Moore, J. E., Jr., Xu, C., Glagov, S., Zarins, C. K., and Ku, D. N. (1994) Atherosclerosis 110, 225–240
13. Sampath, R., Kukiela, G. L., Smith, C. W., Eskin, S. G., and McIntire, L. V. (1995) Ann. Biomed. Eng. 23, 247–256
14. Ohtsuka, A., Ando, J., Kerenaga, R., Kamiya, A., Toyama-Sorimachi, N., and Miyazaki, M. (1995) Biochem. Biophys. Res. Commun. 203, 303–310
15. Davis, M. E., Cai, H., Drummond, G. R., and Harrison, D. G. (2001) Circ. Res. 89, 1073–1085
16. De Keulenaer, G. W., Chappell, D. C., Ishizaka, N., Nerem, R. M., Alexander, R. W., and Griendling, R. K. (1998) Circ. Res. 82, 1094–1101
17. Chappell, D. C., Varner, S. E., Nerem, R. M., Medford, R. M., and Alexander, R. W. (1998) Circ. Res. 82, 522–529
18. Mazzolai, L., Silacci, P., Bouzourene, K., Daniel, F., Brunner, H., and Hayoz, D. (2002) Thromb. Haemost. 87, 1062–1068
19. Silacci, P., Formentin, K., Bouzourene, K., Daniel, F., Brunner, H. R., and Hayoz, D. (2000) Nitric Oxide 4, 47–56
20. Ross, V., Abbott, K. L., Wang, X. F., Pavlath, G. K., and Murphy, T. J. (1998) J. Biol. Chem. 273, 19664–19671
21. Pear, W. S., Nolan, G. P., Scott, M. L., and Baltimore, D. (1993) Proc. Natl. Acad. Sci. U. S. A 90, 8392–8396
22. Schnitzler, H. J., Franke, R. P., Ahkoy, U., Mrowietz, C., and Drenckhahn, D. (1995) Am. J. Physiol. 269, C289–C298
23. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) Nucleic Acids Res. 11, 1475–1499
24. Amati, B., and Land, H. (1994) Curr. Opin. Genet. Dev. 4, 102–108
25. Helmlinger, G., Geiger, R. V., Schreck, S., and Nerem, R. M. (1991) J. Biomech. Eng. 113, 125–131
26. Gallis, B., Corthals, G. L., Goodlett, D. R., Ueha, H., Kim, F., Pressnell, S. R., Figgeys, D., Harrison, D. G., Berk, B. C., Aebersold, R., and Corson, M. A. (1999) J. Biol. Chem. 274, 30101–30108
27. van der Wal, A. C., Becker, A. E., van der Loos, C. M., and Das, P. K. (1994) Circulation 89, 36–44
28. Ip, J. H., Fuster, V., Badimon, L., Badimon, J., Taubman, M. B., and Chesebro, J. H. (1990) J. Am. Coll. Cardiol. 15, 1667–1687
29. Rajagopalan, S., Meng, X. P., Ramasamy, S., Harrison, D. G., and Galis, Z. S. (1996) J. Clin. Invest. 98, 2572–2579
30. Sato, H., and Seki, M. (1993) Oncogene 8, 385–405
31. Yoshizaki, T., Sato, H., Furukawa, M., and Pagano, J. S. (1998) Proc. Natl. Acad. Sci. U. S. A 95, 3621–3626
32. Munaut, C., Salonurmi, T., Kontusaari, S., Reponen, P., Morita, T., Foidart, J. M., and Tryggvason, K. (1999) J. Biol. Chem. 274, 5588–5596
33. Mohan, R., Reinhart, W. B., Bargagna-Mohan, P., and Fini, M. E. (1998) J. Biol. Chem. 273, 25903–25914