Gene Is Programmed by the Tissue Microenvironment

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Abstract. The endothelium is morphologically and functionally adapted to meet the unique demands of the underlying tissue. At the present time, little is known about the molecular basis of endothelial cell diversity. As one approach to this problem, we have chosen to study the mechanisms that govern differential expression of the endothelial cell–restricted von Willebrand factor (vWF) gene. Transgenic mice were generated with a fragment of the vWF gene containing 2,182 bp of 5′ flanking sequence, the first exon and first intron coupled to the LacZ reporter gene. In multiple independent lines of mice, β-galactosidase expression was detected within endothelial cells in the brain, heart, and skeletal muscle. In isogenic transplantation models, LacZ expression in host-derived auricular blood vessels was specifically induced by the microenvironment of the heart. In in vitro coculture assays, expression of both the transgene and the endogenous vWF gene in cardiac microvascular endothelial cells (CMEC) was upregulated in the presence of cardiac myocytes. In contrast, endothelial cell levels of thrombomodulin protein and mRNA were unchanged by the addition of ventricular myocytes. Moreover, CMEC expression of vWF was not influenced by the addition of 3T3 fibroblasts or mouse hepatocytes. Taken together, the results suggest that the vWF gene is regulated by vascular bed–specific pathways in response to signals derived from the local microenvironment.

The endothelium exhibits a remarkable diversity of cellular properties that are uniquely adapted to the needs of the underlying tissue. Heterogeneity within the endothelium has been described at the level of cell structure, antigen composition, mRNA expression, and cell function (Gerritsen, 1987; Kumar et al., 1987; Turner et al., 1987; Tomlinson et al., 1991; Page et al., 1992; Gerritsen and Bloor, 1993). For example, the postcapillary, high venule endothelial cells in lymphoid organs support the binding and migration of lymphocytes via the specific interaction of adhesion molecules with lymphocyte homing receptors (Streeter et al., 1988; Berg et al., 1989; Girard and Springer, 1995). On the other hand, the endothelial cells that line the small blood vessels of the brain possess a unique expression pattern of cell surface receptors, transporters, and intracellular enzymes that serve to tightly regulate the exchange of solutes between blood and brain parenchyma (Bradbury, 1993; Schlosshauer, 1993). Distinct endothelial cell phenotypes have also been documented in other organs such as the liver, kidney, and lung (DeFouw, 1988; Fleming and Jones, 1989). In addition, the endothelium has been shown to vary in its response to pathophysiological stimuli. Escherichia coli–induced sepsis in baboons results in the selective activation of tissue factor in a subpopulation of endothelial cells within the marginal zone of splenic follicles (Drake et al., 1993). In mice, the systemic delivery of lipopolysaccharide results in a specific upregulation of the pentraxin gene family member, ptx-3, specifically within the vascular beds of the heart and skeletal muscle (Introna et al., 1996). These and other examples of the vascular bed–specific endothelial cell response underscore the potential role of phenotypic heterogeneity in mediating focal vasculopathic disease states.

Beyond a large descriptive catalogue of endothelial cell phenotypes, surprisingly little is known about the molecular basis of vascular diversity. An important question that continues to elude us is whether the phenotypic patterns are genetically inherited from distinct sublineages or rather governed by signals residing within the microenvironment. In vitro investigations using embryonic stem cell cultures suggest that endothelial cell differentiation and early vasculogenesis are genetically predetermined (Wang et al., 1992). Retroviral cell tagging studies in chicken em-
bryos have shown different clonal origins for endocardial versus coronary artery endothelial cells (Mikawa and Fischman, 1992). On the other hand, in vivo transplant studies using avian species have pointed to the critical role of environmental cues in establishing blood vessel patterning during development (Poole and Coffin, 1989; Noden, 1990). Unfortunately, these experimental approaches are difficult to adapt to the mammalian system, owing to poor accessibility of embryos and the lack of appropriate cell markers. Nevertheless, there is evidence that regional specialization of the endothelium in mammals may be conditioned by exogenous factors. Perhaps the best examples are found in studies of the blood–brain barrier, in which both in vitro culture and in vivo transplant studies have documented the ability of astrocytes to induce the appropriate phenotype in endothelial cells (Stewart and Wiley, 1981; Beck et al., 1984; Janzer and Raff, 1987; Maxwell et al., 1987; Tao-Cheng et al., 1987; Lobrinus et al., 1992). Additional studies have demonstrated a direct influence of extracellular signals on gene expression in other endothelial cell types. For example, preproendothelin-1 mRNA in rat cardiac microvascular endothelial cells was found to be upregulated when these cells were grown in coculture with venricular myocytes (Nishida et al., 1993). Shear stress has been shown to modulate the transcription of a number of endothelial cell genes through the induction of specific DNA–protein interactions (Resnick et al., 1993; Resnick and Gimbrone, 1995). At this time, it is not clear what role these and other examples of modulatable gene expression play in establishing and/or maintaining the phenotype of a given endothelial cell in the intact animal.

Regardless of the relative roles of clonality and environment in mediating phenotypic differences within the endothelium, the establishment and maintenance of vascular diversity is ultimately controlled at a transcriptional level. Regulation of endothelial cell gene expression has been shown to vary between blood vessel types and vascular beds (Bahnak et al., 1989; Hadley et al., 1994; Kaipainen et al., 1995; Lassalle et al., 1996; Smith et al., 1996). For example, the multimeric glycoprotein von Willebrand factor (vWF), a cofactor for platelet adhesion and a carrier for the antihemophilic factor (for review see Sadler, 1991; Ruggeri and Ware, 1993), is heterogeneously distributed throughout the vascular tree and is associated with regional variations in mRNA levels (Rand et al., 1987; Wu et al., 1987; Bahnak et al., 1989; Coffin et al., 1991; Page et al., 1992; Smith et al., 1996; Aird, W., and R.D. Rosenberg, unpublished results). vWF is expressed at higher levels on the venous side of the circulation compared with arteries and arterioles. By contrast, consistently low levels of vWF are present within the sinusoidal endothelial cells of the liver and spleen. In en face preparations of the rat aorta, expression of vWF appears to vary from one endothelial cell to another (Senis et al., 1996). The gene product is present in clusters of endothelial cells oriented along the longitudinal axis of blood flow and is particularly concentrated in endothelial cells lining the ostia of the intercostal arteries. The administration of thrombin resulted in an increase of histochemically detected vWF expression, suggesting that previously nonexpressing endothelial cells may be recruited to produce vWF (Senis et al., 1996). Taken together, the available evidence suggests that the transcriptional control of vWF varies from one endothelial cell to another and that cell-to-cell variation may be programmed by the extracellular environment. Indeed, an understanding of how these transcriptional networks operate selectively in subsets of endothelial cells should provide an initial framework with which to unravel the molecular mechanisms of differential gene expression and endothelial cell heterogeneity.

Until recently, little was known about the transcriptional regulation of vWF. In transgenic mice, a segment of the human vWF gene containing 487 bp of 5′ flanking sequence, as well as the first exon (+1 to +246) was found to direct expression to a subpopulation of endothelial cells in the adult brain (Aird et al., 1995). These observations suggested that the transgene is under vascular bed–specific transcriptional control and implied that more widespread expression of the vWF gene might be dependent on promoter sequences either proximal to or distal to the 733-bp fragment. To test this hypothesis, a larger segment of the vWF gene containing 2,182 bp of 5′ flanking sequence, the first exon, and the first intron was coupled to the LacZ reporter gene, and the resulting construct (vWFlacZ-2) was used to generate additional lines of transgenic mice. As we report below, transgene expression in these mice was detected not only within blood vessels of the brain but also within the microvasculature of the heart and skeletal muscle. These findings indicate that vWF expression is indeed regulated by distinct organ-specific transcriptional pathways. We also show by transplantation and coculture techniques that cardiac vascular bed–specific control of the vWF transgene and the endogenous gene is modulated by interactions between microvascular endothelial cells and cardiomyocytes. The results support the existence of novel, tissue-specific pathways that regulate the function of endothelial cells in response to signals derived from their local microenvironment.

Materials and Methods

Generation and Analysis of Transgenic Mice

The vWF sequence in vWFlacZ-2 was cloned from a human genomic library (Stratagene, La Jolla, CA). Through sequential cloning steps, the sequence including 2,182 bp of 5′ flanking sequence, the first exon, the first intron, and the translational start site of the human vWF gene was coupled to the SDK sequence. LacZ cDNA and simian virus polyadenylation signal of pSDKlacZpA (generous gift from J. Rossant, Mount Sinai Hospital, Toronto, Canada). The generation and identification of transgenic mice as well as the analysis of tissue sections and whole mounts for LacZ activity and vWF immunohistochemistry were carried out as previously described (Aird et al., 1995). For reverse transcriptase (RT)-PCR, total RNA isolation was isolated from vWFlacZ-2 mouse organs using a guanidinium thiocyanate phenol–chloroform single-step extraction (Stratagene). Approximately 10 μg of total RNA from each organ was treated with DNase and then incubated with reverse transcriptase in the presence of [α-32P]dCTP. First strand cDNA was then used as template for PCR with primer sets specific for E. coli LacZ (5′-GCATCGAGCTGGGTAATAA GCGTTGGCAAT-3′, 5′-GACACCCAGCAACCTGTAATGTTAG CGAC-5′), mouse vWF (5′-ATGATGGAGAGGTTACACATC-3′, 5′ GGCGAGTTGCAGACCCTCCTTG-3′) and mouse thrombomodulin (5′
ACTGATCGGACGCTGCAAGAGTCTGGA 3', 5'-G0CCCGATAT-GTCTCAGATAAGCAATG-3'). The PCR parameters were 95°C for 3 min, 95°C for 45 s and 72°C for 3.5 min, for a total of 40 cycles, followed by 7 min of elongation at 72°C. PCR products were resolved on a 1.2% agarose gel and visualized with ethidium bromide.

Cardiac and Lung Transplantations

Cardiac transplantation experiments were performed as previously described (Rossi, 1992). Briefly, adult vWFlacZ-2 recipient mice were anesthetized with intraperitoneal ketamine, one or both ears were cleaned with 70% ethanol and a subdermal incision 2.5 mm in length was made with a scalpel along the transverse axis of the ear. A pair of microdissection scissors was then used to dissect away intradermal tissue towards the apex of the ear, creating a subdermal ear pouch. The donor heart was removed from the wild-type neonates (12-24-h old) and inserted into the ear pouch. Gentle pressure with delicate curved forceps was then used to express free air from the pocket and to close the incision. Transplanted mice were returned to their cages and cared for according to standard protocols. Neonatal wild-type lung tissue was transplanted into the pinna of the ear of isogenic adult recipients according to a similar protocol. In mock transplant experiments, a subdermal ear pouch was created and then closed as described above without insertion of donor tissue.

In Vitro Coculture Assays

Cardiac muscular endothelial cells (CMEC) were harvested from neonatal and adult mice according to modified protocols (Lodge et al., 1992; Nishida et al., 1993). Adult hearts were excised from anesthetized mice and retrogradely perfused with Hanks' balanced salt solution buffer through the ascending aorta to remove blood cells. The left ventricle was removed aseptically from a total of 8–15 mice between 2 and 5 d of age, and retrogradely perfused with Hanks' balanced salt solution buffer (250 mM Tris-HCl, pH 7.8, 10 mM EDTA). The heart was then minced and placed in ADS buffer (116 mM NaCl, 20 mM Hepes, 1.0 mM NaH2PO4, 5 mM KCl, 0.8 mM MgSO4, 5.5 mM glucose), and minced with a straight-edge razor blade. The tissue was then digested for 45 min at 37°C in DME supplemented with 5% FCS, 0.2% (wt/vol) collagenase and 0.0005% (wt/vol) DNase. The cell-rich supernatant was centrifuged at 200 

Ribonuclease Protection Assays

Template DNA containing sequences from the mouse TM cDNA and from exon 28 of the mouse vWF gene were subcloned into the Bluescript vector (Stratagene). The constructs were linearized by restriction digestion with NotI, and then incubated with T7 in the presence of [32P]UTP to generate single stranded radiolabeled RNA probe. The probe was then hybridized with 20 µg of total RNA in hybridization buffer at 42°C overnight, and the reaction mixture subsequently treated with RNase and proteinase K. The samples were then washed three times with PBS, and incubated with 1% BSA and 0.05% saponin in PBS at room temperature for 1 h. After a wash in PBS, the cells were incubated with vWF-HRP antibody (Dako Corp.) at a dilution of 1:200 for 1 h at room temperature. Cells were washed twice with PBS, and then incubated with 100 µl of solution containing 8 mg orthophenylene-diamine (OPD) substrate (Dako Corp., Carpinteria, CA) in 12 ml 0.1 M citric acid-phosphate, pH 5.0, and 0.0125% H2O2 at room temperature for 3 min. The reaction was stopped with equal volume 1 M sulfuric acid and the A492 was measured by spectrophotometry. For vWF ELISA, the wells were treated with 4% paraformaldehyde in PBS for 15 min on ice and washed three times with PBS, and incubated with 1% BSA and 0.05% saponin in PBS at room temperature for 1 h. After a wash in PBS, the cells were incubated with vWF-HRP antibody (Dako Corp.) at a dilution of 1:200 for 1 h at room temperature. Cells were washed twice with PBS, and then incubated with 100 µl of solution containing 8 mg OPD substrate (Dako Corp.) in 12 ml 0.1 M citric acid-phosphate, pH 5.0, and 0.0125% H2O2 at room temperature for 3 min. The reaction was stopped with equal volume 1 M sulfuric acid, and the A492 was measured by spectrophotometry.

Results

The vWFlacZ-2 Transgene Is Expressed in the Microvascular Bed of the Heart and Skeletal Muscle

The limited pattern of transgene expression in mice harboring a 733-bp fragment of the human vWF gene suggested that DNA sequences outside this region contained information for more widespread endothelial cell expression (Aird et al., 1995). To confirm this speculation, we generated transgenic mice with a larger segment of the vWF gene containing 2,182 bp of 5' flanking sequence, the first exon, and first intron was coupled to the LacZ reporter gene (vWFlacZ-2) (Fig. 1). In seven independent founder lines, the X-Gal reaction product was detected not only within blood vessels of the brain but also in a subset of microvessels within the heart and skeletal muscle (Figs. 2, A–C, and 3). In cardiac sections stained with X-Gal and then processed for immunoperoxidase detec-
tion of endogenous vWF, the transgene and endogenous gene products colocalized in the endothelial lining of capillary vessels (Fig. 3B). In contrast, the endothelial cells of the coronary arteries, coronary veins, penetrating arteries, and endocardium of the heart exhibited no detectable β-galactosidase activity but possessed immunoreactive vWF (Fig. 3A, arrowhead, absence of LacZ staining in epicardial coronary artery). Transgene expression was similarly absent in the vascular bed of other organs, including the liver, spleen, lung, and kidney, as well as in the aorta and megakaryocyte lineage (Fig. 2B–F). In each of the seven lines of mice, ectopic reporter gene activity was detected within a subpopulation of neurons within the hypothalamus and cerebellum (data not shown). In RT-PCR analyses, LacZ mRNA was detected only in brain, heart, and skeletal muscle (Fig. 4). In contrast, mRNA from the endogenous vWF gene and from the endothelial cell-restricted TM gene was present in all tissues examined. vWF mRNA levels varied from one organ to another (Fig. 4) and correlated with transcript levels detected by ribonuclease protection assays (data not shown). Thus, the above vascular bed–specific expression pattern of vWF-LacZ-2 provides further evidence that the vWF transgene is regulated through the interaction of regional transcriptional networks with distinct promoter elements.

**Wild-type Heart Grafts Induce Transgene Expression in Endothelial Cells Derived from LacZ-negative Blood Vessels**

We then wished to determine the relative importance of environmental cues and genetic factors in programming expression of the vWF-LacZ-2 transgene in different vascular beds. To investigate this issue, we used a syngeneic cardiac transplantation model in which neonatal donor hearts are grafted into the ear of adult recipient mice (Rossi, 1992). In these experiments, wild-type neonatal were harvested within 24 h of birth, and immediately implanted under the subdermal layer of the ear pinna of adult vWF-LacZ-2 transgenic mice. The blood vessels of the host ear rapidly vascularize the graft (Fig. 5A), and endothelial cells from this nonexpressing bed are newly exposed to a myocardial environment. The grafts were analyzed between 3–6 wk after transplantation when the functional viability of the implanted organ was confirmed both by visible pulsations and electrocardiographic activity (Fig. 5B). In six independent transplants, LacZ activity was detected in numerous blood vessels surrounding ventricular myocytes (Fig. 5C and D). The X-Gal reaction product was not observed in the vascular bed of the ear proper, nor within the blood vessels of mock-transplanted transgenic littermates (data not shown). LacZ staining was also absent in neonatal lung tissue transplanted into the pinna of six vWF-LacZ-2 transgenic mice (Fig. 5F). As a control for neovascularization, wild-type lungs were also grafted into a transgenic mouse that contains β-galactosidase activity in endothelial cells of every vascular bed including the ear (Aird, W.C., and R.D. Rosenberg, unpublished observations). In these mice, an abundance of LacZ-positive blood vessels within the substance of the graft indicate that the transplanted lung is revascularized by host-derived endothelium (Fig. 5B–D).
Bar, 26 μm.

Figure 3. The vWFlacZ-2 transgene colocalizes with endogenous vWF within the microvessels of the heart. (A) Whole mounts of the vWFlacZ-2 adult heart incubated with the X-Gal substrate reveals diffuse LacZ staining in both ventricles and atria with distinct sparing of the epicardial coronary arteries (arrowhead). (B) 10-μm section through the left ventricular wall of the vWFlacZ-2 adult heart processed for β-galactosidase activity (blue) and immunoperoxidase detection of vWF (black) reveals co-localization (arrowheads) within the endothelial lining of the microvessels. Bar, 26 μm.

E). Taken together, these results suggest that certain vascular beds outside the heart, skeletal muscle, and brain retain the competence to express the vWFlacZ-2 transgene in response to the microenvironment of the heart. In other words, the critical information required for cardiac microvascular-specific transcriptional activation of the vWF transgene is not contained within the endothelial cell, but rather within the surrounding myocytes or the extracellular milieu. These in vivo observations support the view that organ-specific endothelial cell gene expression is ultimately controlled by the interplay between local environmental factors and intracellular transcriptional networks.

Cardiac Myocytes Induce Expression of Both Transgene and Endogenous vWF under In Vitro Coculture Conditions

We next established a coculture system to delineate the interaction between CMEC and ventricular myocytes involved in transgene activation. To this end, neonatal or adult transgenic CMEC were harvested and grown under in vitro conditions. After 4 d of culture, pure populations of CMEC no longer exhibited LacZ activity (data not shown). However, when overlaid with wild-type ventricular myocytes, CMEC reacquired the X-Gal reaction product. Interestingly, most LacZ-positive endothelial cells occurred within clusters of spontaneously beating ventricular myocytes (Fig. 6B). To quantitate the extent of transgene induction, LacZ activity was assayed in whole plate lysates with the ONPG substrate. On average, β-galactosidase activity in primary cultures of CMEC (120 U/10^5 cells) was 2.6-fold higher in cocultures of CMEC and myocytes as compared to CMEC alone (Fig. 6A). Of importance, the total numbers of endothelial cells, as monitored by cell-specific markers, failed to increase upon the addition of ventricular myocytes which argues against a proliferative effect of the coculture conditions (data not shown). Moreover, the antigenic levels of the endothelial cell marker TM were not elevated, suggesting that the inductive process is specific for β-galactosidase (Fig. 6A). Furthermore, cocultures of CMEC with BNL hepatocytes or 3T3 fibroblasts failed to induce LacZ expression (data not shown). Thus, the above data suggest that maintenance and reinduction of the vWFlacZ-2 transgene in CMEC are specifically mediated by cardiac myocytes.

Finally, we asked whether endothelial cell expression of the endogenous vWF gene and the vWFlacZ-2 transgene is controlled in a similar fashion by cardiomyocytes. Primary cultures of CMEC between passages 1–4, exhibited barely visible vWF antigen as judged by immunohistochemical staining (data not shown). By comparison, strongly positive vWF-containing endothelial cells were readily discernible within clusters of ventricular myocytes under coculture conditions (Fig. 6C). Total cellular vWF antigenic levels in CMEC (1 ng/10^5 cells) were increased by an average of 3.1-fold upon addition of cardiomyocytes (Fig. 6A), but were not altered in the presence of mouse hepatocytes or 3T3 cells (data not shown). To exclude the possibility that a translational mechanism was responsible for elevating endogenous vWF concentrations, vWF mRNA levels were determined by ribonuclease protection assays under coculture conditions. Primary cultures of CMEC ex-
Figure 5. Environmental induction of transgene expression in cardiac transplantation model. (A) Whole mount photomicrograph of a 3-wk-old neonatal, wild-type cardiac graft in the ear of an adult vWFlacZ-2 mouse showing the complex network of anastomosing host auricular blood vessels. (B) Two-lead electrocardiogram of a transplanted heart revealing electrocardiographic activity. The heart rate of the graft was 150 beats per min, compared with the native heart rate of 320 beats per min under anesthesia. (C) X-Gal staining of a thick 100-μm section from the cardiac graft reveals the presence of β-galactosidase activity in a linear pattern. (D) X-Gal staining of an 8-μm section from the cardiac graft reveals the presence of LacZ-containing endothelial cells next to wild-type ventricular myocytes. (E) X-Gal staining of a 12-μm section through a wild-type lung graft transplanted into the ear of a transgenic mouse that expresses LacZ in all vascular beds. The presence of LacZ-positive blood vessels indicates that the lung graft is revascularized by host-derived endothelium. (F) X-Gal staining of a 12-μm section through a wild-type lung graft in the ear of a vWF-lacZ-2 mouse ear revealing absence of detectable LacZ activity. Bars: (C,E,F) 60 μm; (D) 12 μm.

Figure 6. Protein expression in cardiac microvascular endothelial cell-ventricular myocyte coculture. (A) β-Galactosidase activity in CMEC from vWFlacZ-2 mice, as measured with the ONPG substrate, was 2.6-fold higher under coculture conditions (CMEC + myo) compared with either vWF-lacZ-2 or wild-type CMEC alone. Antigenic levels of cellular vWF were stimulated 3.1-fold under similar conditions. In contrast, there was no change in the antigenic levels of the endothelial cell marker TM when CMEC was coplated with ventricular myocardial cells. The results are derived from at least three independent experiments, each performed in triplicate. Protein levels are calculated relative to values obtained from primary cultures of vWFlacZ-2 and wild-type CMEC. (B) X-Gal staining of a coculture plate containing CMEC and cardiomypocytes reveals the presence of numerous LacZ-positive endothelial cells integrated within a cluster of myocytes. (C) vWF immunofluorescence under coculture conditions reveals a similar staining pattern with strongly positive endothelial cells interspersed within a colony of cardiomyocytes. Bar, 100 μm.
hhibited decreased concentrations of transcript as compared to whole heart or freshly harvested CMEC (Fig. 7). The addition of ventricular myocytes to CMEC resulted in a threefold induction of vWF expression (Fig. 7). In contrast, TM mRNA levels did not change significantly (Fig. 7).

**Discussion**

In the present study, a region of the human vWF gene encompassing 2,182 bp of 5′ flanking sequence, the first exon and first intron coupled to the coding region of LacZ was used to generate transgenic mice. In seven independent lines of mice, reporter gene activity and mRNA were limited to the endothelial lining of blood vessels in the brain, heart and skeletal muscle, indicating that this particular promoter fragment contains information sufficient for vascular bed–specific expression of vWF. The limited distribution of the transgene contrasts with the more widespread expression of the endogenous gene and suggests that alternative mechanisms of transcriptional activation are operative in LacZ-negative endothelial cells. These observations add vWF to a growing list of endothelial cell subpopulations within transgenic embryos (Schlaeger et al., 1995). In a similar study, a 735-bp region of the mouse Tie-1 gene was shown to confer endothelial cell subtype-restricted expression in transgenic mice (Harats et al., 1995). In one report, DNA promoter constructs containing either 1,200 or 600 bp of the murine Tie-2 promoter were shown to direct expression to distinct endothelial cell subpopulations within transgenic embryos (Schlaeger et al., 1995). In a similar study, a 735-bp region of the mouse Tie-1 gene was shown to confer endothelial cell subtype-specific expression during development (Korhonen et al., 1995). In contrast, activity of the Tie-1 and Tie-2 transgenes was downregulated in adult mice (Korhonen et al., 1995; Schlaeger et al., 1995). Finally, a 5.9-kb fragment of the murine preproendothelin-1 promoter directed expression within the endothelium and vascular smooth muscle cells of adult transgenic mice (Harats et al., 1995). Expression levels in these mice varied not only between arteries, veins, and capillaries, but also between vascular beds of different organs (Harats et al., 1995). Taken together, these studies provide strong support for the existence of regional differences in the mechanisms of endothelial cell gene regulation.

The existence of cell subtype-specific mechanisms of gene regulation is not limited to the endothelium. In transgenic mice, the α1(1) collagen gene was shown to possess different cis elements required for expression in fibroblasts of the skin as compared to fibroblasts within the fas-

![Figure 7](image-url) Changes in vWF antigen correlate with transcript levels. In ribonuclease protection assays, total RNA from freshly harvested heart (heart), CMEC and CMEC in coculture with ventricular myocytes (CMEC + myo) was hybridized to riboprobes specific for mouse vWF, TM, and β-actin mRNA.
of these pathways might underlie the focal nature of vascular diseases.

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