tions, could be solubilized by Triton X-100, and was stable in the solubilized extracts.

The CS protein gene is representative of the *Plasmodium* surface antigens that have been cloned and characterized so far. These include the CS protein of *P. knowlesi* (*5*) and *P. falciparum* (*20, 21*), the S-antigen (*13*), the ring-infected erythrocyte surface antigen (*22*), and the pPF11-1 antigen (*23*) of *P. falciparum*. Each of these surface antigens contains many copies of a highly conserved, tandemly repeated peptide unit made of 4 to 12 amino acids. In the case of the CS proteins, where the complete genes have been isolated and sequenced, the repeated peptide units constitute ~40 percent of the polypeptide chain (*5, 20*). These units were found to be the most immunogenic parts of these proteins, as almost all monoclonal antibodies isolated against the sporozoite were directed against the repeated epitope (*2*). As has been pointed out (*22*), the repeats may mask an immune response directed against other surface antigens or against a different hapten on the same molecule. With the purification of the CS protein it will be possible to study the second hypothesis, namely whether peptide sequences other than the repeated units would make a more effective protective antibody than the repeated polypeptide or the entire protein.

SHOBHONA SHARMA
G. NIGEL GODSON
Department of Biochemistry, New York University Medical Center, New York 10016

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Modulation of the *sis* Gene Transcript During Endothelial Cell Differentiation in Vitro

Abstract. *Endothelial cells, which line the interior walls of blood vessels, proliferate at the site of blood vessel injury. Knowledge of the factors that control the proliferation of these cells would help elucidate the role of endothelial cells in wound healing, tumor growth, and arteriosclerosis. In vitro, endothelial cells organize into viable, three-dimensional tubular structures in environments that limit cell proliferation. The process of endothelial cell organization was found to result in decreased levels of the* sis *messenger RNA transcript and increased levels of the messenger RNA transcript for fibronectin. This situation was reversed on transition from the organized structure to a proliferative monolayer. These results suggest a reciprocity for two biological response modifiers involved in the regulation of endothelial cell proliferation and differentiation in vitro.*

The abluminal surface of the vascular tree consists of a nonthrombogenic monolayer of endothelial cells that functions as a metabolic interface between blood and tissue (*1*). Damage to the vessel wall signals the endothelial cell to migrate and divide at the site of injury (*1*), events critically important for maintenance of the structural integrity of the vascular system. Indeed, neovascularization contributes significantly to controlling the growth of tumors, the maximum size of which would otherwise be limited by diffusion rate (*2*). Thus, characterization of the factors controlling endothelial cell division is important in determining the role of the endothelial cell in atherogenesis, wound healing, and tumor growth.

Human endothelial cells isolated from diverse blood vessels have been successfully propagated in vitro (*3*). The growth of human endothelial cells in vitro is controlled by endothelial cell growth factor (ECGF), a potent polypeptide mitogen isolated from bovine brain (*4*). Withdrawal of ECGF from the human endothelial cell monolayer results in a rearrangement of the cells within the monolayer into viable three-dimensional tubular structures that have a lumen and thus resemble microvessels (*5*). Although the precise polarity of the tubular structures is unknown, the relative ease with which human endothelial cells may be grown in vitro and manipulated so as to form organized structures readily lends itself to the study of the molecular biology of this phenomenon.

We used Northern blot analysis to study gene expression in proliferating and organized human endothelial cells. Because human endothelial cells produce a platelet-derived growth factor (PDGF)–like mitogen (*6*), and c-*sis* is considered to be the gene encoding for the larger of the two heterodimeric chains of PDGF (*7*), we analyzed the intracellular content of PDGF messenger RNA (mRNA) with nick-translated v-*sis* complementary DNA (cDNA) as a probe. In parallel experiments, we examined fibronectin mRNA content. Fibronectin is a structural component of the extracellular matrix and is the mediator of endothelial cell migration and attachment to collagen (*1, 5, 8*). Furthermore, fibronectin is invariably involved in the process of endothelial cell organization (*5*).

Endothelial cells from human umbilical vein were grown in fibronectin-coated plastic roller bottles in Medium 199 supplemented with fetal bovine serum and ECGF (Fig. 1A). For induction of capillary tube formation, cells from confluent monolayers were collected by treatment with trypsin and reintroduced into the original culture vessels (*5*). The cells that were exposed to the trypsin-treated human endothelial cell matrix were then incubated without ECGF in medium that was changed weekly. One week after removal of ECGF, we observed profound changes in the morphology of the cells in the newly established monolayer. At this stage the endothelial cells had begun to migrate and to organize into aligned clusters (Fig. 1B). After prolonged culture (5 weeks) in the ab-

sence of ECGF (Fig. 1C), the aligned clusters of cells had organized into an elongated and interconnected tubular network. At this stage, the cells were harvested from the organized structures with trypsin and collagenase and introduced into the ECGF-supplemented cell culture system. The single cell suspension of human endothelial cells reverted to the proliferative phenotype as demonstrated by a rapid increase in cell number and the formation of a stable endothelial cell monolayer (*5*). These observations confirm our original observation that endothelial cell organization represents a nonterminal state of differentiation in vitro (*5*). We used this culture system to examine the effects of nonterminal differentiation on gene regulation in human endothelial cells.

Total RNA was isolated by a slight modification of the method of Chirgwin *et al.* (*9*) from in vitro human endothelial cell populations (legend to Fig. 1). For each sample, total RNA was fractionated by electrophoresis in an agarose-formaldehyde gel and transferred to GeneScreen Plus (New England Nuclear) essentially as described by Thomas (*10*). Hybridization of the total RNA with nick-translated v-*sis* cDNA revealed a single transcript species of approximately 4.3 kb (relative to ribosomal RNA size markers) (Fig. 2B). This band is also weakly present in total RNA preparations obtained from a proliferative population of a rat promegakaryoblast cell line (*11*). Additional experiments also showed a band of the same size in total RNA isolated from A172 glioma cells, which produce relatively large quantities of PDGF-like polypeptide (*12*). The size of the band is similar to that of the c-*sis* gene transcript (*13*). Human endothelial cells in the process of organization as a result of extracellular matrix modification and mitogen withdrawal (*5*) showed approximately 50 percent reduction in the amount of c-*sis* transcript (Fig. 2B, lane d). Percentages are based on densitometric analyses. After 5 weeks of continuous culture in the absence of ECGF, approximately 80 percent of the cells were organized into a tubular network, and the c-*sis* transcript was approximately 23 percent of the control value (Fig. 2B, lane e). Finally, treatment of the organized endothelial cell population with trypsin and collagenase followed by subculture in the presence of ECGF reversed the organizational pattern of human endothelial cells obtained when ECGF was absent. The process of endothelial cell reversion resulted in an increase in the PDGF mRNA content (Fig. 2B, lane f) to levels approaching those of the original endothelial cell monolayer. These results are consistent with the observation that the *sis* gene may be activated in the endothelial cell as a result of the transition from its position in the vessel wall in vivo to an established primary monolayer culture (*14*).

To determine whether the results observed with v-*sis* could be due to differences in RNA integrity or perhaps to unequal loading of samples, we prepared a duplicate Northern blot and probed it with a human cDNA fragment specific for fibronectin (*15*). A prominent band, approximately 7.8 kb in size, was observed in all endothelial cell RNA preparations. However, the levels of the fibronectin transcript also varied as a function of endothelial cell differentiation. Whereas the c-*sis* transcript decreased in organizing endothelial cells, the fibronectin transcript increased by more than 40 percent (Fig. 2A, lanes c and d). This increase was not maintained in fully organized endothelial cells (Fig. 2A, lane e), nor was it observed in the revertant endothelial cell monolayer derived from the organized tubular structures. These findings presumably reflect an increased requirement and rate of synthesis of fibronectin—and perhaps other extracellular matrix components—by endothelial cells during the process of organization.

There appears to be a reciprocal relation between the proliferative capacity of human endothelial cell populations and the ability of human endothelial cells to organize into structures morphologically

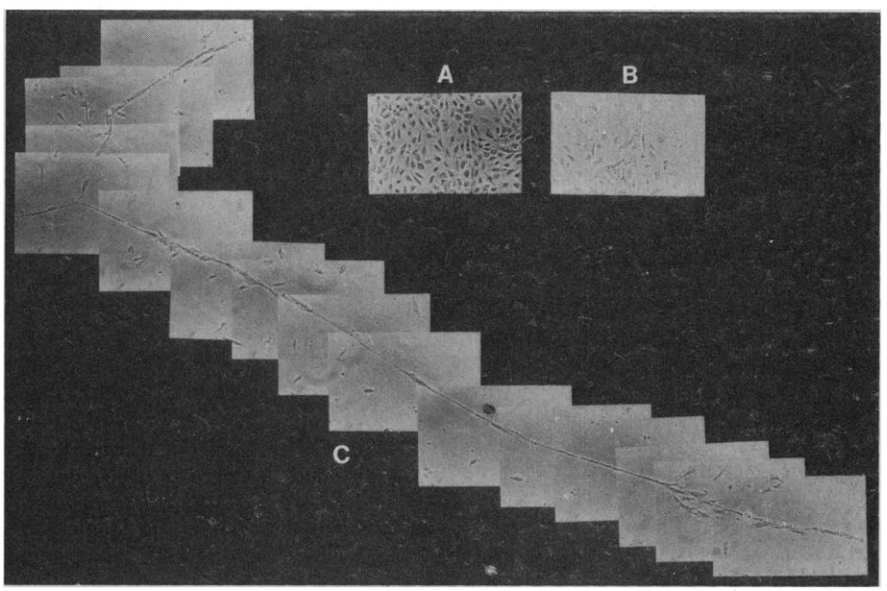

Fig. 1. Morphological changes apparent during human endothelial cell organization. Human umbilical vein endothelial cells (HUVEC) were grown to confluence in 850-cm^2 plastic roller bottles coated with human fibronectin (10 μg/cm^2) in Medium 199 supplemented with 10 percent fetal bovine serum and 150 μg of a crude preparation of bovine brain ECGF per milliliter prepared as previously described (*22*). The process of endothelial cell organization was initiated by treatment of the HUVEC monolayer with 0.05 percent trypsin and 0.02 percent EDTA. The single cell suspension was then introduced into the recycled trypsin-treated roller bottles (20 bottles) at a split ratio of 1:5. These cultures were incubated with Medium 199 supplemented with 10 percent fetal bovine serum in the absence of ECGF. The medium (100 ml) was changed every 7 days. Extensive tubular networks of HUVEC were observed after 5 weeks in culture. Every random field of vision contained at least one organized HUVEC network. Revertant populations of HUVEC were obtained by treatment of the organized HUVEC population as follows. Bottles containing tubular networks were treated with 0.05 percent trypsin and 0.02 percent EDTA. After inhibition with soybean trypsin inhibitor (200 μg/ml), the structures were further treated with 0.05 percent collagenase (Worthington) for 30 minutes at 37°C. The entire single cell suspension was seeded into a roller bottle coated with human fibronectin (10 μg/cm^2). The cells were supplemented with Medium 199 containing fetal bovine serum and ECGF as described above. The HUVEC population proliferated to confluence as an attached cobblestone monolayer (*5*). (A) Phase-contrast morphology of confluent HUVEC monolayer (magnification, ×250); (B) appearance of HUVEC population 1 week after extracellular matrix recycling in the absence of ECGF. The cobblestone morphology is lost and the cells have migrated into an aligned site for the initiation of the organizational process (magnification, ×250). Populations of similar HUVEC structures were collected for RNA isolation and identified as "organizing" or stage II morphology (*5*). (C) Organized network of HUVEC after 5 weeks in culture (magnification, ×250). There is an extensive tubular network with branch points. Populations of similar HUVEC structures were collected for RNA isolation and labeled as "organized" or stage IV morphology (*5*). Revertant populations of HUVEC derived from stage IV populations are not shown but have a similar if not more compact morphology than observed in (A).

resembling a differentiated phenotype. The ability of the endothelial cell to produce a potent polypeptide growth factor (*6*) that may be a signal for smooth muscle cell division is inversely related to the expression of an extracellular matrix signal that may participate in endothelial cell migration (*8*) and differentiation (*5*). This is consistent with the observations (*5, 16*) that the process of endothelial cell organization is favored in environments that limit endothelial cell proliferation. It also seems likely that changes in the expression of c-*sis* and fibronectin genes may occur in association with additional alterations in the phenotypic expression of the endothelial cell, perhaps in senescence and in sprouting endothelial cell populations (*5*).

The human endothelium in situ is a rather quiescent monolayer (*17*). Complex biological events and biochemical signals, such as endothelial injury or angiogenesis factors, are capable of inducing neovascularization, which includes localized endothelial cell proliferation (*1, 2*). Our demonstration of an increase in the c-*sis* transcript in proliferating as opposed to organized endothelial cells suggests that endothelial cell–derived PDGF-like proteins may be important in neovascularization. Our results also provide evidence that endothelial cell–derived PDGF-like activity is the product of the human c-*sis* gene (*6, 14*).

The precise role of the PDGF-like molecule in neovascularization is not clear. PDGF is a potent chemotactic polypeptide for monocytes and neutrophils as well as fibroblasts and smooth muscle cells (*18*). Thus PDGF, and perhaps the c-*sis* gene product, have the potential to attract inflammatory cells and stimulate smooth muscle cell and fibroblast migration and proliferation at concentrations of PDGF well below those found in human serum (*18*). It therefore appears likely that during the development of the neovasculature the c-*sis* gene product may act as a paracrine biological response modifier of mesenchymal cell migration and division. Furthermore, it is possible that in endothelial cell malignancy, such as angiosarcoma, there is excessive transcription of the c-*sis* gene and that the expression of c-*sis* during endothelial cell proliferation has a role in the pathogenesis of atherosclerosis (*6, 14*). The endothelial cell–derived PDGF-like molecule, a potent vascular smooth cell mitogen (*6*), may independently contribute to or act in concert with PDGF deposited by the platelet after endothelial cell injury.

The addition of PDGF to BALB/c 3T3 cells in vitro rapidly induces the expression of several genes including c-*myc* (*19*), the cellular homolog of the transforming gene of avian myelocytomatosis virus MC29 (*20*). These results would be relevant to the expression of c-*myc* in proliferative populations of human endothelial cells if endothelial cell–derived PDGF were capable of stimulating endothelial cell division in an autocrine manner. However, PDGF does not stimulate human endothelial cell division in vitro (*3*), and endothelial cells do not contain high-affinity binding sites for PDGF (*21*). Thus a study of *myc* gene expression as a function of endothelial cell organization may provide additional information about the molecular events relevant to the developmental biology of the vasculature.

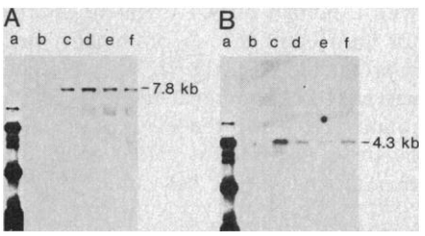

Fig. 2. Northern blot analysis of total RNA from HUVEC at several states of organization. Total RNA from cultured rat promegakaryoblast (*11*) and HUVEC at several states of organization was denatured in 2.2*M* formaldehyde and 50 percent formamide and fractionated by electrophoresis in a 1.25 percent agarose gel containing 2.2*M* formaldehyde. This was transferred to GeneScreen Plus (New England Nuclear) by blotting with 10× SSPE (SSPE is 0.18*M* NaCl, 0.10*M* $NaPO_4$, and 1 m*M* EDTA). (A) Blots were hybridized to ^{32}P-labeled nick-translated probes of fibronectin cDNA at 65°C for 16 hours in a mixture containing 2× SSPE, 20× Denhardt's solution (Denhardt's solution is 0.02 percent bovine serum albumin, 0.02 percent polyvinylpyrrolidone, and 0.02 percent Ficoll 400), yeast transfer RNA (200 μg/ml), and 0.2 percent sodium dodecyl sulfate (SDS). A 1.1-kb Eco RI subfragment of the fibronectin cDNA clone (HF771), corresponding to 200 base pairs of the sequence coding for fibronectin and 900 base pairs of 3′ untranslated sequence, was used as a hybridization probe. The membrane was subsequently washed at 65°C, twice with 2× SSPE and 0.2 percent SDS, then twice with 0.2× SSPE and 0.2 percent SDS, air-dried, and exposed overnight to Kodak XAR film with an intensifying screen. (Lane a) DNA standards; (lane b) rat promegakaryoblast; (lane c) HUVEC as a confluent monolayer; (lane d) HUVEC as an organizing population of cells harvested after at least one tube was present in the roller bottle during cell culture; (lane e) HUVEC harvested after completion of the organization process into tubular networks of cells, which are apparent in every field of vision in the roller bottle during cell culture; (lane f) HUVEC revertant as a confluent monolayer derived from trypsin treatment of the organized cell population (Fig. 1C), as in lane e. Lanes b to f contained 2.7 μg of total RNA. (B) The lane assignments are identical to those in (A) except that each lane contained 8.02 μg of total RNA. A 1.2-kb cDNA fragment of simian sarcoma virus containing 1 kb of v-*sis* cDNA fused to a 0.2-kb *env* gene cDNA from simian sarcoma virus was contributed by K. Robbins. Exposure with Kodak XAR was for 14 days, although faint bands were visible after shorter exposures. The quality and quantity of each RNA preparation was assessed by ethidium bromide staining of ribosomal RNA.

MICHAEL JAYE
EVELYN MCCONATHY
WILLIAM DROHAN
Department of Molecular Biology, Meloy Laboratories, Revlon Health Care Group, Springfield, Virginia 19014
BENTON TONG
THOMAS DEUEL
Departments of Medicine and *Biological Chemistry, Jewish Hospital of St. Louis, Washington University, St. Louis, Missouri 63110*
THOMAS MACIAG*
Department of Cell Biology, Revlon Biotechnology Research Center, 2501 Research Boulevard, Rockville, Maryland 20850

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* To whom correspondence should be addressed.
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Matrix-Driven Translocation of Cells and Nonliving Particles

Abstract. *Cells of metazoan organisms produce and react to complex macromolecular microenvironments known as extracellular matrices. Assembly in vitro of native, compositionally nonuniform collagen-fibronectin matrices caused translocation of certain types of cells or polystyrene-latex beads from regions lacking fibronectin into regions containing it. The translocation process was not due to diffusion, convection, or electrostatic distribution effects, but may depend on nonequilibrium phenomena at the interface of contiguous collagen matrices formed in the presence and absence of fibronectin or particles. Extracellular matrix formation alone was sufficient to drive translocation by a biophysical process that may play a role in cellular migration during embryogenesis, as well as in other types of tissue reorganization such as inflammation, wound healing, and tumor invasion.*

Extracellular matrix components such as the collagens, fibronectin, laminin, hyaluronic acid, and proteoglycans influence cellular biosynthetic activities, shapes, and motility during interactions with one another and with different types of cells (*1*). It has usually been considered that cellular translocation results from forces generated within cells that are interacting with their matrices (*2*). The matrix would impart speed and direction to this motile activity by a combination of chemokinetic (*3*), chemotaxic (*4*), and route-opening (*5*) effects. However, cells with no intrinsic motile behavior, and even nonliving particles such as polystyrene beads, can be selectively conveyed along neural crest pathways in embryos into which they have been artificially introduced (*6*). We now report that matrices constructed from collagen and fibronectin generate a driving force that is capable of translocating living cells and nonliving particles several millimeters in a matter of minutes.

Chick embryo cells of various types were mixed with a gelling solution of type I collagen (*7, 8*). Contiguous with the cell-containing gel was a second gel (legend to Fig. 1), poured at the same time and containing different fibronectins (3 to 50 μg/ml). At an appropriate fibronectin concentration in the second gel (in excess of a threshold value between 3 and 6.25 μg/ml), the cells moved as much as 6 mm into the originally cell-free region in about 10 minutes. The rate of translocation (defined as movement over distances of at least 1 mm) varied with the concentration of collagen but not with that of fibronectin once its threshold level had been reached.

Primary gels containing chick limb bud precartilage mesenchyme cells (*9, 10*) (2.5×10^6 cells per milliliter) were formed adjacent to secondary gels containing chick cellular, chick plasma, or human plasma fibronectin (12.5 μg/ml), or no fibronectin. Translocation began 10 to 15 seconds after the gels were poured, and continued for as much as 20 minutes. Over a period of 5 to 20 minutes, cells from the border regions, including those from about 1 mm away from the interface, moved into the secondary gels containing chick cellular or human plasma fibronectin, with the farthest moving cells traversing more than 5 mm. Movement then stopped and no further change was observed in 2 days of observation. In the presence of any of several biologically active preparations of chick plasma fibronectin (*11*) or in the absence of fibronectin, no translocation took place during the 2-day period (displacement of the cell front from the original boundary was less than 0.25 mm). This was the case when concentrations of chick plasma fibronectin were more than eight times the threshold level for chick cellular or human plasma fibronectin (Fig. 1). These experiments were repeated 30 to 50 times for each fibronectin, with six separately prepared batches of rat tail tendon collagen, and there was no variation in the qualitative results. The rate of translocation depended on the collagen concentration. If the collagen concentration was higher or lower than the optimal value of about 1.7 mg/ml, translocation of cells was slower or did not occur.

Individual cells and groups of cells translocated at speeds of more than 5 μm/sec (Fig. 2). Furthermore, the path taken by each moving cell was unidirectional, with none of the random walk characteristics that would be expected for a stochastic process such as diffusion. Cells did not move in opposite directions.

Different cell types varied in their responses to matrices containing different fibronectins. Chick limb bud precartilage cells translocated in response to human plasma fibronectin and chick cellular fibronectin, but not in response to chick plasma fibronectin. Chick embryo fibroblasts responded to the human fibronectin, but not to either chicken fibronectin. In contrast, cells obtained from hearts of 5-day chick embryos did not respond to any of the fibronectins (*12*).

Two observations suggested that the translocating cells were not moving under their own power. (i) The observed rates of translocation were more than an order of magnitude greater than the most rapid cellular movement previously described in vitro (*2*); (ii) cells translocating in collagen gels showed none of the changes in cellular morphology associated with motile behavior, as assessed by Nomarski differential interference contrast microscopy.

Polystyrene latex beads are conveyed along neural crest pathways in embryos by a driving force arising from the extracellular environment (*6*). We therefore introduced such beads, instead of cells, into collagen gels as the primary gel systems (Fig. 3, a and b). When human plasma fibronectin was present in the secondary gel there was massive translocation of the beads; when it was absent there was no translocation. The same threshold concentration of fibronectin (between 3 and 6.25 μg/ml) was necessary here as in the cell-containing system. The beads were not translocated in response to chick plasma fibronectin, and only to a small extent with chick