Role of CL-100, a Dual Specificity Phosphatase, in Thrombin-induced Endothelial Cell Activation*

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Using a cDNA microarray screening approach, we have identified seven novel thrombin-responsive genes in human umbilical vein endothelial cells that were verifiable by Northern blot analysis. Among them CL-100, a dual-specificity phosphatase also known as MAP kinase phosphatase-1 (MKP-1), showed greatest induction by thrombin. Steady-state levels of CL-100 mRNA induction by thrombin peaked at 1 h and declined rapidly (t1/2 ~45 min). Induction by thrombin was protease-activated receptor-1-mediated, protein synthesis-independent, and transcriptionally regulated. Metabolic labeling followed by immunoprecipitation verified that the thrombin-induced CL-100 mRNA was translated into protein. We found that both Src-kinase and p42/p44 ERK activity are critical for thrombin-induced CL-100 expression, whereas phosphatidylinositol 3-kinase and protein kinase C activity were not required. Antisense-mediated inhibition of CL-100 was shown to prolong thrombin-induced ERK activity in endothelial cells, concomitant with an inhibition in thrombin-induced PDGF-A (platelet-derived growth factor A) and PDGF-B gene expression and an up-regulation in thrombin-induced VCAM-1 and E-selectin gene expression. Inhibition of ERK activation by PD98059 in endothelial cells was shown to potentiate thrombin-induced expression of PDGF-B (~3-fold) while inhibiting thrombin-induced VCAM-1 and E-selectin gene expression by 60 and 70%, respectively. These results suggested that induced expression of the CL-100 phosphatase and its subsequent regulation of ERK activity play a key regulatory role in the thrombin signaling pathway and in the transcriptional regulation of pathologically important “endothelial cell activation genes.”

Thrombin, a multifunctional serine protease, plays a critical role in hemostasis, coagulation, and thrombosis. In addition, thrombin can function as an agonist for cellular responses in a variety of cell types, including endothelial cells (EC)1 (1). Thrombin mediates most of its cellular responses through activation of seven transmembrane-spanning G protein-coupled receptors known as PARs (protease-activated receptors). Three thrombin receptors, PAR-1, PAR-3, and PAR-4, have been identified so far in mammalian cells (1, 2). Recent studies suggest that PAR-1 is the predominant thrombin receptor expressed in human umbilical vein EC (3). Thrombin activates PAR-1 by proteolytic cleavage at a unique site within the amino terminus of the receptor. Receptor cleavage exposes a new amino terminus that serves as a tethered ligand by binding to sites within the body of the receptor, which results in receptor activation (4, 5). Synthetic peptides corresponding to the amino terminus of the tethered ligand can function as a thrombin receptor agonist peptide (TRAP) and mimic thrombin activation of receptors (4, 6). In EC, thrombin receptor activation triggers the induction of a variety of molecules, including PDGF-A, PDGF-B, and cell adhesion molecules such as E-selectin, VCAM-1, and ICAM-1 that are known to play a central role in vascular development and vascular injury (7, 8). In many cases, the signaling pathways mediating the thrombin induction of such EC activation genes are complex and not well delineated. In an attempt to identify novel thrombin-inducible second messengers and their putative target genes, we screened ~5000 genes using a cDNA microarray technique (Research Genetics, AL). We identified seven novel thrombin-inducible genes that were subsequently confirmed independently by Northern blot analyses to be reproducibly induced by thrombin. We focus in this report on one such gene, CL-100, a dual specificity phosphatase that was the most robustly induced in response to thrombin and that had the greatest potential for mediating expression of downstream thrombin-inducible genes.

CL-100 is a human homologue of MAP kinase phosphatase-1 (MKP-1). CL-100 belongs to a family of inducible nuclear dual specificity phosphatases that catalyze dephosphorylation of both serine/threonine and tyrosine residues efficiently (9). CL-100 is an immediate early gene induced in various cell types by oxidative stress, heat shock, UV light, and growth factors (10, 11). It is known to dephosphorylate specific threonine and tyrosine residues in kinases of the MAPK pathway, such as ERK, stress-activated protein kinase, and c-Jun NH2-terminal kinase, resulting in their inactivation (12). However, the MKP-1 family of phosphatases appears to exhibit cell type specificity in choosing their substrates (9, 13, 14), and their role in regulating endothelial cell function is not very well established. Recently, involvement of CL-100 in mediating oxidized phospholipid-induced monocyte chemotactic activity in EC has been reported (15). It was postulated that CL-100 was required for oxidized phospholipid-induced monocyte chemoattractant protein-1 production, although the mechanism of this regulation was not elucidated. In another study, Potente et al. (14) demonstrate that inactivation of c-Jun NH2-terminal kinase by CL-100 phosphatase activity is critically important in ep-
oxeyeico-satrinneio acid-induced cyclin D1 up-regulation and, subsequently, EC proliferation.

Thrombin couples to multiple G proteins (Gαs, Gαi, Gα12, Gα13, and Gαq) and activates various MAP kinases, including ERK, through several pathways (1, 16–19). These signaling pathways ultimately lead to the activation of transcription factors that mediate the induction of a variety of genes important for EC function. However, the specific role of MAPK components in mediating transcription of these specific EC genes is poorly understood. In this study, we have demonstrated that thrombin activation of EC results in the induction of the CL-100 phosphatase gene. We have further shown that CL-100, through its regulation of the ERK signaling pathway, regulates both positively and negatively thrombin-induced transactivation of specific EC activation genes.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—EC were isolated by trypsin digestion of human umbilical veins as described before (20). Isolated EC were maintained in MCD/F12 medium (Sigma) containing 15% fetal bovine serum, 0.009% heparin, 100 ng/ml aprotinin, and 200 μM phenylmethylsulfonyl fluoride. Inosoluble material was removed by centrifugation. Cell lysates were incubated with agarose-conjugated anti-MKP-1 (M-18: sc-1102 AC; Santa Cruz Biotechnology) antibody for 2 h at 4 °C. The immunocomplex was washed three times with lysis buffer, solubilized in 1× Laemmli buffer, resolved in 10% SDS-PAGE, and detected using a Storm phosphorimaging system as described above.

**Nuclear Run-on Assay**—Nuclei were isolated by cell lysis in 0.5% NP-40 or extracted from thrombin-treated or untreated EC using 200 μg/ml aprotinin and 100 μg/ml leupeptin, 20 μg/ml soybean trypsin inhibitor, and 1 mM phenylmethylsulfonyl fluoride.

**RESULTS**

Identification of Seven Novel Thrombin-responsive Genes by Microarray Analysis.—In an attempt to identify novel thrombin-inducible genes in human EC, we employed a cDNA microarray approach (GF201; Research Genetics) allowing for the rapid analysis of nearly 5000 genes. Two independent screens reproducibly showed up-regulation of many known genes as well as seven additional genes that were not previously known to be induced by thrombin. The induction of these seven genes was subsequently verified and validated by Northern blot analysis (Table I). The results of both the microarray and Northern
MRNA levels were induced to induce specific to PAR-2, a non-thrombin receptor expressed in EC, failed PAR-4-specific peptide AYPGKF or peptides SLIGKV or SLIGRL elicited in response to 1 and 5 units/ml of thrombin (Fig. 2). We identified that PAR-1-specific agonistic peptide (TRAP-1; SFLLRNP) receptor(s) responsible for EC (3). We used receptor-specific agonistic peptides to identify the receptor(s) responsible for PAR-1 induction by thrombin. We identified that PAR-1-specific agonistic peptide (TRAP-1; SFLLRNP) induced CL-100 in human umbilical vein EC (Fig. 2a). CL-100 mRNA levels were induced 4-fold in response to 5 units/ml of thrombin or 100 μM of SFLLRNP. CL-100 mRNA levels induced in response to 100 and 200 μM of SFLLRNP were similar to those elicited in response to 1 and 5 units/ml of thrombin (Fig. 2b). PAR-4-specific peptide AYPGKF or peptides SLIGKV or SLIGRL specific to PAR-2, a non-thrombin receptor expressed in EC, failed to induce CL-100 induction (data not shown).

**CL-100 Induction Is Independent of de Novo Protein Synthesis and Regulated at Transcriptional Level**—To test whether the induction of CL-100 by thrombin is dependent on de novo protein synthesis, we treated EC with cycloheximide (10 μg/ml) 30 min prior to thrombin stimulation and determined CL-100 mRNA levels by Northern blot analysis. As shown in Fig. 3a, cycloheximide treatment had little effect on thrombin-induced CL-100 gene induction, suggesting that newly synthesized proteins were not required to mediate the thrombin effect. To determine whether thrombin-induced CL-100 mRNA levels were the result of an increase in the transcriptional rate and/or a reduction in mRNA decay, EC were pretreated with the transcription inhibitor actinomycin D (10 μg/ml) prior to thrombin stimulation. As shown in Fig. 3b, actinomycin D substantially inhibited thrombin-induced CL-100 gene expression, resulting in near basal levels of expression.

We also examined, by performing an actinomycin decay curve, the relative stability of CL-100 mRNA isolated from untreated and thrombin-treated EC and did not observe any significant differences. The half-life of the CL-100 in both cases was ~45 min (data not shown). We further confirmed that thrombin mediated the de novo transcription of the CL-100 gene by performing nuclear run-on transcription assays. As shown in Fig. 4, run-on assays from thrombin-treated EC nuclei revealed a consistent and greater than 2.5-fold increase in the transcription rate of the CL-100 gene in response to thrombin.

**Induced CL-100 mRNA Is Translated into Protein in EC**—To verify that the induced CL-100 mRNA is translated into protein, we determined relative CL-100 protein levels in response to thrombin induction. To accomplish this, we metabolically labeled EC with [35S]methionine and cysteine (Translabel) and subjected cells to actinomycin D (100 μg/ml) and stimulated cells with TRAP-1 for the times indicated. Cellular lysates were immunoprecipitated with antibody M18 and analyzed by SDS-PAGE and autoradiography. As demonstrated in Fig. 5, actinomycin D substantially inhibited thrombin-induced CL-100 protein expression, resulting in near basal levels of expression.

### Table 1

| Gene Bank Accession Number | Gene Name | Fold Induction | Northern Blot Analysis |
|---------------------------|-----------|----------------|------------------------|
| AA703141                  | Protein 4.1 | 1.71/1.59     | 1.30                   |
| H87536                    | Bullous pemphigoid auto antigen | 1.80/1.50 | 1.20                   |
| N67048                    | 3-isothyronine deiodinase | 1.72/1.52 | 1.15                   |
| 783257                    | TIP-1-like protein | 1.57/2.01 | 1.25                   |
| AA682851                  | ERP-31     | 1.80/2.07     | 1.25                   |
| X68277                    | CL-100     | 1.67/1.36     | 1.90                   |
| AA703141                  | Troponin T2 | 1.76/1.48     | ND                     |

**Novel thrombin-inducible genes in EC identified by microarray analysis**

Human umbilical vein EC were exposed to thrombin (5 units/ml for 2 h), and total RNA was isolated. Two gene microarray screens were performed on two independent sets of RNAs (experiments 1 and 2) from thrombin-treated or untreated (control) EC. RNA from experiment 1 was used for Northern blot analyses. ND, not determined.
in the presence and absence of thrombin by Western blot analysis. However, multiple commercially available CL-100/MKP-1 antibodies failed to detect endogenous CL-100 in human umbilical vein EC lysates. Because CL-100 is an "immediate early gene" having a short protein half-life (45 min) and TRAP-1 treatment of EC resulted in newly synthesized CL-100 protein (Fig. 5), we assumed there is a transient increase in CL-100 protein levels upon TRAP-1 or thrombin treatment.

Transcriptional Regulation of CL-100 by Thrombin Is MAPK-dependent and Pertussis Toxin-insensitive—Because MAPK is a downstream component in the regulation of many PAR-1-inducible genes (22–26), we next determined whether MAPK mediates thrombin-induced CL-100 gene expression. Pretreatment of EC with the selective p42/p44 ERK inhibitor PD-98059 inhibited by greater than 90% TRAP-1-induced CL-100 gene expression (Fig. 6), whereas p38 kinase inhibitor SB203580 was without effect (data not shown). It is known that upstream of the MAPK pathway the PAR-1 receptor of thrombin is coupled to both pertussis toxin-sensitive and -insensitive G proteins (5, 27). In an attempt to determine whether thrombin-induced CL-100 is mediated through a Gi family member does not couple the PAR-1 receptor to downstream CL-100 gene induction.

Thrombin-induced CL-100 Gene Expression Is Src Kinase-dependent and PKC- and PI3-kinase-independent—It is generally accepted that thrombin receptor PAR-1 activation of the MAPK pathway in EC is mediated through two major signaling pathways. One is mediated through βγ-subunits of G proteins...
that activate non-receptor tyrosine kinases such as Src, which interacts with and phosphorylates the Grb2-SOS complex and through regulation of p21ras mediates MAPK activation. In the other pathway, PAR-1 activation results in the activation of phospholipase C by G proteins, leading to phosphoinositide hydrolysis, Ca²⁺ mobilization, diacylglycerol formation, and PKC activation. PKC phosphorylates Raf kinase, bypassing p21ras, which stimulates MAPK activation (5). Because we have demonstrated that p42/p44 ERK activity is required for thrombin-induced CL-100 gene expression (Fig. 6), we next tested the potential role of the upstream activators of ERK activity in our response. As shown in Fig. 7, pretreatment of EC for 30 min with the specific Src kinase inhibitor PP1 (10 μM) completely inhibited TRAP-1-mediated induction of CL-100 gene expression. A similar pretreatment with PKC inhibitor GF109203 (10 μM) or PI3-kinase inhibitor LY294002 (10 μM) had no significant effect on TRAP-1-induced CL-100 mRNA levels. Thus, thrombin-induced CL-100 gene expression requires Src kinase activity and is independent of PKC and PI3-kinase activity.

CL-100 Mediates Thrombin-induced Gene Expression in EC—Because CL-100 induction by thrombin is an early event, we explored the possibility that CL-100 activity is required for mediating the thrombin-induced expression of various endothelial cell activation genes in EC. We addressed this question by applying both pharmacological and antisense oligonucleotide-mediated approaches. First, we investigated the effect of the Src inhibitor, PP1, which completely blocked thrombin-induced CL-100 gene expression (Fig. 7), on thrombin induction of various genes in EC using real-time PCR. As shown in Table II, PDGF-A and PDGF-B gene induction by thrombin was completely inhibited by a 30-min pretreatment of EC with PP1 (10 μM). Interestingly, PP1 pretreatment did not inhibit but rather potentiated thrombin-induced expression of E-selectin and VCAM-1 mRNA (2- to 3-fold). PP1 had no effect on the induction of ICAM-1 by thrombin (Table II).

We next addressed the regulatory role of CL-100 in thrombin-induced gene expression more directly using specific antisense oligonucleotides to inhibit CL-100 expression in EC. We based the design of our oligo sequences on a previously published report (15) (5'-CCCACCCTTCCATGACCATGG-3'); however, we modified the transfection protocol to render this approach amenable to our experimental condition. We avoided the use of the SuperFect reagent because it adversely affects thrombin activation of EC. Instead, we treated EC with the oligonucleotides in the absence of any transfecting reagent. As can be seen in Fig. 8, this approach resulted in the complete inhibition of the steady-state levels of CL-100 mRNA induced by thrombin. The random control oligonucleotide did not significantly inhibit thrombin-induced CL-100 mRNA levels (Fig. 8). We next measured the expression of EC activation genes in the presence of the CL-100-specific antisense oligonucleotide following a 3-h thrombin treatment of EC by using quantitative real-time PCR (Table III). Similar to the results of Table II with the Src kinase inhibitor PP1, antisense oligonucleotides directed against CL-100 completely inhibited thrombin-induced PDGF-A and PDGF-B mRNA steady-state levels. In the case of thrombin-induced E-selectin and VCAM-1 gene expression, we observed a modest increase in mRNA levels (20 and 40%, respectively) following CL-100 antisense treatment.

Inhibition of ERK Activation Mimics CL-100 Induction in the Expression of Thrombin-inducible EC Activator Genes—p42/p44 ERK and p38 kinases have been shown to be activated within minutes following thrombin treatment and are known to be involved in endothelial cell activation, proliferation, and migration (2, 25, 26, 28). CL-100 is known to dephosphorylate and thereby inactivate both ERK and p38 kinase activity. Therefore, we postulated that the effect of CL-100 in the regulation of EC activation genes is mediated through its modulation of ERK and/or p38 kinase activity. If this were the case, by inhibiting the activity of ERK and/or p38 we would expect a pattern of gene expression that was the reverse of that observed with CL-100 inhibition in thrombin-stimulated EC. Thus, we pretreated EC with the ERK inhibitor PD98059 or the p38 inhibitor SB203580 prior to thrombin stimulation and analysis of EC activator genes by real-time PCR (Table IV). In agreement with our hypothesis, we observed a nearly 3-fold increase in thrombin-induced PDGF-B mRNA steady-state levels, whereas E-selectin and VCAM-1 mRNA steady-state levels
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Real-time PCR (Q-PCR) assay using gene-specific primers on RNA (2 μg) isolated from thrombin-treated (3 h) human umbilical vein EC in the presence of CL-100 antisense or random oligonucleotide. Q-PCR was performed along with RPL-32 as housekeeping gene (internal control) to ensure quality and efficiency of the PCR. All the Q-PCR data reported showed single amplicon, and the authenticity of each amplicon was verified by DNA sequencing. Each reaction was performed in triplicate for all the samples. Comparative quantification, based on cycle threshold of the gene of interest, was normalized to RPL-32.

| Genes   | Thrombin alone | Thrombin and random oligo | Thrombin and antisense oligo |
|---------|----------------|---------------------------|-----------------------------|
| PDGF-A  | 39.1 ± 5.2     | 33.5 ± 3.8                | 2.4 ± 1.0                   |
| PDGF-B  | 5.3 ± 1.5      | 4.6 ± 1.2                 | 1.8 ± 0.9                   |
| E-selectin | 530 ± 38.5   | 465.4 ± 26                | 758.1 ± 55                  |
| VCAM-1  | 1234 ± 128     | 834.5 ± 140               | 1522.9 ± 112                |
| ICAM-1  | 8.6 ± 2.4      | 6.7 ± 1.8                 | 5.5 ± 1.2                   |
| CL-100a | 14.1 ± 3.4     | 19.1 ± 4.6                | 2.5 ± 0.9                   |

Thrombin treatment for 1 h.

| Genes | Fold induction (mRNA) |
|-------|-----------------------|
| PDGF-A| 28 ± 4.4              |
| PDGF-B| 2.5 ± 0.6             |
| E-selectin | 164 ± 24.1 |
| VCAM-1| 3.2 ± 1.4             |
| ICAM-1| 7.5 ± 2               |

Human umbilical vein EC were grown to confluency and treated with PD98059 (ERK inhibitor) or SB203580 (p38 inhibitor) in serum-free medium for 30 min. Cultures were then treated with thrombin (5 units/ml) for 3 h. Cells were lysed and total RNA isolated as described under “Experimental Procedures.” RNA (2 μg each) were subjected to real-time PCR (Q-PCR) assay using gene-specific primers. Q-PCR was performed along with RPL-32 as housekeeping gene (internal control) to ensure quality and efficiency of the PCR. All the Q-PCR data reported showed single amplicon, and the authenticity of each amplicon was verified by DNA sequencing. Each reaction was performed in triplicate for all the samples. Comparative quantification, based on cycle threshold of the gene of interest, was normalized to RPL-32.

**DISCUSSION**

We used cDNA microarray analysis to identify novel thrombin-inducible genes in EC. Our microarray analysis from two independent experiments identified seven novel thrombin-inducible genes that are implicated in a wide range of biological functions. Among them, CL-100, a dual specificity phosphatase, showed robust induction at both the mRNA and protein levels. Thrombin induction of CL-100 in EC is transient; both CL-100 mRNA and protein expression peaked at 1 h and then decayed rapidly to basal levels (t½ ~45 min). We found that Src-kinase activity and p42/p44 ERK activity were critical for thrombin-induced CL-100 expression. CL-100 inhibition resulted in down-regulation of thrombin-induced PDGF-A and PDGF-B mRNA expression, whereas VCAM-1 and E-selectin mRNA levels were increased upon CL-100 inhibition.

Thrombin-induced signaling in EC results in a number of phenotypic changes, including changes in cell shape, permeability, migration, proliferation, and induction of leukocyte adhesion (7, 29–34). Many of these phenotypic changes are mediated through the induction of newly synthesized genes. The thrombin receptor PAR-1 is predominantly expressed in EC; its function has been demonstrated to be required for the induction of many of these newly synthesized genes (2). In this study, we have demonstrated that thrombin induction of CL-100 is mediated solely through PAR-1 and is initiated through coupling to a pertussis toxin-insensitive G protein. In EC, thrombin-induced pertussis toxin-insensitive G protein coupling has been shown to induce both ERK and p38 MAPK activation. We have shown herein that ERK activity is critical for thrombin-mediated CL-100 mRNA induction. This is in contrast to earlier studies in non-endothelial cell types where PKC and p38 were shown to be required for the induction of CL-100 by various agents including LPS, LDL, arsinite, UV radiation, oxidative stress, or hypoxia (35–38).

In EC, it has previously been shown that PAR-1 activation by thrombin can transactivate the EGF receptor (39, 40). Such indirect receptor tyrosine kinase activation, in turn, results in ERK activation. We ruled out such an indirect activation scenario in our thrombin-induced CL-100 gene induction because inhibition of P13-kinase activity, a critical component for EGF transactivation by PAR-1 signaling, did not inhibit CL-100...
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induction. Furthermore, blocking EGF kinase activity with the AG1478 inhibitor had no effect on thrombin-induced CL-100 gene induction. Thus, we conclude that EGF receptor transactivation is not required for thrombin-induced CL-100 in EC. We propose instead that PAR-1 activation by thrombin results in the activation of the Src family of tyrosine kinases that we have shown are required for thrombin-mediated CL-100 gene expression. Because we have demonstrated that PKC is not involved, Src most likely recruits the adaptor complex Shc-Grb-Sos that in turn activates RAS proteins (5, 6). Activated RAS, through Raf, results in MEK activation and subsequent phosphorylation and activation of ERK. Downstream targets of activated ERK may include kinases, transcription factors, histones, and elongation factors, each of which is implicated in CL-100 induction by various agonists in different cell types (35–37, 41, 42). Currently we are investigating the role of such downstream molecules in thrombin-mediated CL-100 gene induction in EC.

The MAP kinase signaling pathway has been shown to play a critical role in thrombin-induced gene expression and EC activation. The CL-100 phosphatase has recently been shown to regulate a variety of cellular functions through its modulation of the MAP kinase signaling pathway (43, 44). For example, Liu et al. (45) report an increase in erythropoietin gene expression upon CL-100 inhibition in HepG2 cells and demonstrate that this was a result of increased phosphorylation and transactivation of the transcription factor HIF-1. In another report, Reddy et al. (15) demonstrate a positive regulatory role of CL-100 on monocyte chemoattractant protein-1 protein production and EC monocyte chemotactic activity in oxidized phospholipid-activated EC. In this report, we have identified that CL-100 serves both positive and negative regulatory roles in thrombin-mediated gene expression. We demonstrated that CL-100 activity is required for the induction of both PDGF-A and PDGF-B gene expression, whereas it negatively regulates the thrombin-induced expression of the cell adhesion molecules E-selectin and VCAM-1. We have also presented evidence that demonstrates that this regulatory role of CL-100 in modulating thrombin-induced gene expression is mediated through its downstream regulation of ERK activity. Inhibition of CL-100 activity by pharmacological inhibitors or by antisense approaches leads to a deregulation of ERK phosphorylation with a concomitant loss in normal thrombin-mediated gene expression in EC. Thus, we propose that the CL-100 phosphatase, through its regulation of ERK, acts as a “regulatory switch” in the mediation of differential gene expression in thrombin-treated EC.

Our study has also demonstrated that ERK activity, and not p38 kinase activity, plays a predominant role in thrombin-mediated gene induction in EC. Persistent ERK activity in the absence of CL-100 mediates a negative regulatory role in the induction of two PDGF molecules, whereas ERK activity mediates a positive regulatory role in mediating E-selectin and VCAM-1 expression by thrombin. However, in agreement with earlier reports by others, we found that TNF-α-mediated induction of E-selectin and VCAM-1 in EC requires p38 kinase activity (Table IV) (46, 47). Furthermore, we have shown that inhibition of ERK activity results in a consistent increase (~2-fold) in TNF-α-mediated E-selectin gene expression. This observation suggests the importance of maintaining a delicate balance and cross-talk between the various branches of the MAP kinase signaling cascade. We believe the CL-100 phosphatase, through its ability to modulate the various branches of this signaling pathway, represents a critical regulatory molecule in mediating thrombin-mediated gene expression in EC.

In summary, we have demonstrated a novel mechanism for regulation of thrombin signaling and gene expression in EC. Our results suggest that CL-100 plays a critical role in mediating differential gene expression in thrombin-treated EC via both positive and negative regulation of genes such as PDGF-B, E-selectin, and VCAM-1. CL-100 mediates such a differential regulatory role by virtue of its ability to modulate thrombin-induced ERK activation. We propose that the CL-100 phosphatase acts as a regulatory control switch in mediating the expression of thrombin-inducible EC activation genes.

Acknowledgments—We thank P. Hoang and L. Mavrakis for cell culture assistance. Human umbilical vein endothelial cells were harvested through the Birthing Services Department at the Cleveland Clinic Foundation and the Perinatal Clinical Research Center (National Institutes of Health Research Center Award RR-00080) at the Cleveland Metropolitan Hospital.

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J. Biol. Chem. 2004, 279:46678-46685.
doi: 10.1074/jbc.M406441200 originally published online August 31, 2004

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