Comparison of Promoters for the Murine and Human P-selectin Genes Suggests Species-specific and Conserved Mechanisms for Transcriptional Regulation in Endothelial Cells*

(Received for publication, November 13, 1997, and in revised form, February 12, 1998)

Junliang Pan, Lijun Xia, and Rodger P. McEver‡

From the Departments of Medicine and Biochemistry & Molecular Biology, W. K. Warren Medical Research Institute, University of Oklahoma Health Sciences Center, and Cardiovascular Biology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma 73104

P-selectin, an adhesion receptor for leukocytes, is constitutively expressed in megakaryocytes and endothelial cells. Tumor necrosis factor-α (TNF-α) or lipopolysaccharide (LPS) increases synthesis of P-selectin in murine but not in human endothelial cells. To identify potential species-specific and conserved mechanisms for regulation of expression of P-selectin, we cloned the 5′-flanking region of the murine P-selectin gene and compared its features with those previously reported for the human gene. The murine and human genes shared conserved Stat-like, Hox, Ets, GATA, and GT-IH elements. In the murine gene, a conserved GATA element bound to GATA-2 and functioned as a positive regulatory element, whereas a conserved Ets element bound to GA-binding protein and functioned as a negative regulatory element. Significantly, the murine P-selectin gene had several features not found in the human gene. These included an insertion from −987 to −649 that contained tandem GATA and tandem AP1-like sequences, which resembled enhancers in β-globin locus control regions. Both tandem elements bound specifically to nuclear proteins. The murine gene lacked the unique κB site specific for p50 or p52 homodimers found in the human gene. Instead, it contained two tandem κB elements and a variant activating transcription factor/cAMP response element site, which closely resembled sites in the E-selectin gene that are required for TNF-α or LPS-inducible expression. TNF-α or LPS augmented expression of a reporter gene driven by the murine, but not the human, P-selectin promoter in transfected endothelial cells. Deletional analysis of the murine 5′-flanking region revealed several sequences that were required for either constitutive or inducible expression. These data suggest that both species-specific and conserved mechanisms regulate transcription of the human and murine P-selectin genes.

Leukocyte extravasation is mediated by the sequential engagement of adhesion molecules, whose expression and/or function are regulated by inflammatory mediators (1–3). For example, tumor necrosis factor-α (TNF-α)1 or LPS stimulates endothelial cells to transcribe the genes encoding the adhesion molecules E-selectin, intercellular cell adhesion molecule-1, vascular cell adhesion molecule-1, and mucosal addressin cell adhesion molecule-1 (2, 4–9). These newly synthesized proteins are directly transported to the cell surface, where they mediate adhesion of leukocytes. In contrast, the expression of P-selectin is regulated by two distinct mechanisms. First, P-selectin is constitutively synthesized by megakaryocytes (the precursors of platelets) and endothelial cells, where it is sorted into the membranes of secretory granules (10–14). When these cells are stimulated by agonists such as thrombin, P-selectin is rapidly translocated from granules to the plasma membrane (15), where it initiates tethering and rolling of leukocytes (16–19). Second, inflammatory mediators such as TNF-α, LPS, interleukin-4, or oncostatin M increase levels of P-selectin mRNA and protein in endothelial cells (20–22). The increased synthesis of P-selectin may saturate the sorting pathway into secretory granules, leading to direct delivery of the protein to the cell surface (22, 23). Notably, TNF-α or LPS increases expression of P-selectin in murine endothelial cells but not in human endothelial cells (22, 24). The mechanism for this unusual species-specific response is not known.

We previously isolated and conducted a preliminary analysis of the 5′-flanking region of the human P-selectin gene (25, 26). The sequence from −309 to −13 relative to the translational start site conferred tissue-specific expression of a reporter gene in cultured bovine aortic endothelial cells. Deletions or mutations of this sequence revealed at least three positive regulatory regions that included a κB site, a GATA element, and at least two potential Ets elements. Most κB sites are recognized by inducible homodimeric or heterodimeric NF-κB/Rel proteins that participate in cytokine-stimulated gene expression. However, the κB site in the human P-selectin promoter bound specifically to constitutively expressed homodimers of p50 or p52, whose functions were differentially regulated by the proto-oncogene product, Bcl-3 (26). GATA and Ets elements are often observed in genes whose expression is restricted to hematopoietic and/or endothelial cells (25, 27–34). The human P-selectin GATA element bound to the protein GATA-3 (25, 27–34). The human P-selectin GATA element bound to the protein GATA-3 (25), and mutation of this element markedly inhibited gene expression (25). The function of the potential Ets elements in the P-selectin gene was not determined.

To better understand potential conserved and nonconserved mechanisms for expression of the P-selectin gene, we isolated the 5′-flanking region of the murine P-selectin gene and com-

1 The abbreviations used are: TNF-α, tumor necrosis factor-α; bp, base pair(s); BAEC, bovine aortic endothelial cells; GABP, GA-binding protein; LPS, lipopolysaccharide; PCR, polymerase chain reaction.
pared its features with those previously reported for the human gene (25, 26). Both genes share functional GATA and Ets sites. However, the murine gene has several unique putative regulatory elements that may explain why TNF-α or LPS augments transcription of the P-selectin gene in murine but not in human endothelial cells.

**EXPERIMENTAL PROCEDURES**

**Cells, Reagents, and Antibodies—**Murine bEnd.3 endothelial cells, BAEC, and human megakaryocytic HEL and CHRP-288 cells were cultured as described (22, 25). Recombinant human TNF-α and yeast RNA were obtained from Boehringer Mannheim. LPS from *Salmonella typhosa* was purchased from Sigma. Antibodies against human GATA-2, Ets-1, Ets-2, Fli-1, and p65 (RelA) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

**Genomic Cloning, Southern Blot Analysis, and Sequence Comparison—**To clone the murine P-selectin 5′-flanking region, we designed the following two primers: 5′-ACATTCTCTGAAACCGAATAAGC-3′ which spanned nucleotides 1–22 of the murine P-selectin cDNA sequence, and 5′-CATGCTCTCTGCTCCTGCT-3′ which is complementary to nucleotides 105–126 of the cDNA sequence (21). Based on the known human P-selectin gene structure (35), we predicted that the murine P-selectin 5′-untranslated region and the translation initiation ATG would be encoded by one exon. We confirmed this prediction by sequencing the PCR product amplified from mouse genomic DNA using the above two primers. The primers were then used in a PCR screen to identify bacteriophage P1 clones encoding the murine 5′-flanking region (custom service provided by Genome Systems, Inc., St. Louis, MO). The PCR profile involved denaturation at 94 °C for 1.5 min, primer annealing at 50 °C for 2.5 min, and extension at 72 °C for 1 min, with a final concentration of Mg2+ at 1.5 mM. Two P1 clones named GS512 and GS5572 were obtained. DNA restriction fragments derived from the GS5512 clone were subcloned into the plasmids pBluescript II SK (Stratagene) or pBluescript (IBI) for restriction enzyme mapping and DNA sequencing. Southern blot analysis of mouse genomic DNA with a probe of the sequence.

**RESULTS**

**Molecular Cloning of the 5′-Flanking Region of the Murine P-selectin Gene—**Two mouse P1 clones encoding the murine P-selectin gene were obtained using PCR screening. Southern blot analysis of these two clones revealed the same restriction patterns as those obtained with mouse genomic DNA, suggesting that there was no DNA rearrangement during cloning (data not shown). Restriction fragments derived from the P1 clone GS5512 were subcloned into plasmids (Fig. 1). The insert in subclone pMG2 was sequenced on both strands; it contained the 1256 bp of the 5′-flanking region, all of exon 1, and part of intron 1.

**Determination of the Transcription Start Site—**We employed an RNase protection assay to determine the transcription start site of the murine P-selectin gene. Several protected products were seen from −134 to −75 relative to the ATG translation start codon (Fig. 2). These protected products were observed in mRNA from LPS/cycloheximide-stimulated bEnd.3 cells but not in control yeast tRNA. The longest product was the most abundant. These data indicate that transcription of the murine P-selectin gene is initiated primarily at −134 relative to the translation start site.

**Comparison of Structural Features of the Murine and Human 5′-Flanking Regions—**Fig. 3 aligns the 5′-flanking sequence of the murine P-selectin gene with the corresponding sequence of the human gene (25). There was 63% sequence identity between the two genes in the first 600 bp of the 5′-flanking region, exon 1, and the first 500 bp of intron 1. This degree of similarity is typical for the mouse and human homologues of a gene (40). Both genes ended the first exon with the translation start codon ATG and shared several putative regulatory elements that may explain why TNF-α or LPS augments transcription of the P-selectin gene in murine but not in human endothelial cells.
Promoters of Murine and Human P-selectin Genes

Several unique aspects of the murine P-selectin gene were noted. First, unlike the human P-selectin gene (25), the murine gene initiated transcription predominantly at a single site, consistent with the presence of a weak TATA box (−160 A/GTTATA-CAA−153) located 21 bp upstream of this start site. The weak TATA box overlapped the conserved GATA element. We referred to this murine sequence as a GATA/TATA element because of its possible dual functions. Second, the murine gene contained an insertion from −875 to −862, which resembled the enhancers in the DNAse I hypersensitive sites of the β-globin locus control region (46). Third, the murine gene lacked the unique κB site and surrounding putative regulatory elements previously identified in the human gene (25, 26). Instead, it contained a κB site in reverse orientation from −144 to −136 and an AP1 element from −262 to −256 (47, 48). It also had two tandem κB elements from −542 to −521 and a variant activating transcription factor/cAMP sequence from −80 to −73 that resembled those required for cytokine-inducible expression of the human and murine E-selectin genes (49–52).

The GATA/TATA Element Is Required for Optimal Murine P-selectin Promoter Activity—To test whether the murine P-selectin GATA/TATA element bound to nuclear proteins, we synthesized double-stranded oligonucleotide probes encompassing the wild-type sequence or a mutant sequence in which the core TATC was converted to TTAG (Fig. 4A). As a positive control, we used a human endothelin-1 GATA probe that is known to bind specific GATA proteins (25, 53, 54). The labeled P-selectin probes did not form detectable sequence-specific DNA-protein complexes. However, a 100-fold molar excess of the unlabeled wild-type probe, but not the mutant probe, weakly competed with the human endothelin-1 GATA element for binding to GATA proteins (Fig. 4B). These data suggest that the GATA/TATA element interacts weakly with GATA proteins.

To determine whether the GATA/TATA element was required for promoter activity, we made a construct (mp1379Luc) in which the murine 5′-flanking region from −1379 to −13 was linked to luciferase. We also made a mutant construct in which the TATCA of the wild-type sequence was converted to TTAG, the same changes made in the mutant probe used for the gel shift assay. When transfected into BAEC, the mutant construct was expressed at levels ~75% lower than those of the wild-type sequence (Fig. 4C). These results indicate that the GATA/TATA element is essential for optimal transcription of the murine P-selectin gene.

Nuclear Protein Binding Activities of Tandem GATA Motifs and Tandem AP-1-like Elements in the Insertion—As illustrated in Fig. 5A, the murine P-selectin sequence from −923 to −849 contained tandem GATA and tandem AP-1-like elements, which closely resembled the enhancers in hypensensitive sites 2 and 3 of the murine β-globin locus control region (46).

We used gel shift assays to determine whether the tandem elements bound to nuclear proteins. A labeled probe encoding the tandem GATA elements formed a DNA-protein complex with nuclear extracts from bEnd.3 cells (Fig. 5B). Formation of the complex was sequence-specific, as addition of a 100-fold molar excess of the unlabeled probe, but not an unrelated oligonucleotide, suppressed formation. Complex formation was dependent on the GATA core sequence, because a labeled probe with mutations introduced into tandem GATA elements did not bind nuclear proteins. The unlabeled human P-selectin and endothelin-1 GATA probes also competed with the murine tandem GATA probe for complex formation. Preincubation of HEL cell nuclear extracts with antibodies to GATA-2 diminished binding of the labeled tandem GATA probe to nuclear proteins, whereas antibodies to Est-1/Est-2 or to the κB protein Flk-1 had no effect (Fig. 5C). These data indicate that the tandem GATA elements bound to members of the GATA protein family.

A labeled probe encompassing the tandem AP-1-like elements formed a DNA-protein complex with nuclear extracts from bEnd.3 cells (Fig. 5D). Complex formation was sequence-specific, as formation was suppressed by addition of a 100-fold molar excess of the unlabeled probe but not of an unlabeled murine H-2Kb κB recognition element (55). These data indicate that the region encompassing tandem AP-1-like elements also forms a sequence-specific DNA-protein complex.
tides encoding the murine and human proximal Ets sequences (Fig. 6A). A labeled probe encoding the murine proximal Ets element formed two DNA-protein complexes with nuclear extracts from BAEC (Fig. 6B) and from bEnd.3, HEL, and CHRF-288 cells (data not shown). Formation of these two complexes was sequence-specific, as it was significantly diminished by addition of a 100-fold excess of the unlabeled probe, or of the proximal Ets element from the human gene, but not of an unrelated sequence. Complex formation further depended on the Ets core sequence, because a mutant probe in which the core sequence AGGAAG was converted to AGCTAG did not inhibit binding of the labeled wild-type probe and, when labeled, did not form specific DNA-protein complexes (Fig. 6C).

Of note, formation of complex I varied with different batches of nuclear extracts, as previously noted for binding of the Ets-related GABP to its cognate DNA sequences (56). We therefore carried out cross-competition gel shift assays with the proximal Ets probes and the HSV1 IE Ets probe that interacts with GABP (56). Addition of a 50-fold excess of the HSV1 IE Ets probe, but not a 200-fold excess of an unrelated sequence, prevented complex formation of the labeled proximal murine P-selectin Ets probe with BAEC nuclear extracts (Fig. 6D). The labeled HSV1 IE Ets probe formed three DNA-protein complexes with nuclear extracts from BAEC (Fig. 6E) or from HEL, CHRF-288, or bEnd.3 cells (data not shown). However, only complex A was consistently observed. Formation of complex A was prevented or significantly diminished by addition of a 50–200-fold excess of the unlabeled HSV1 IE Ets element or the murine or human proximal Ets probe but not of the mutant proximal Ets probes. These data suggest that the proximal Ets elements bind to one or more members of the Ets family, including GABP.

To determine the potential function of the murine proximal Ets element, we changed the proximal Ets sequence AGGAAG to AGCTAG in the mp1379LUC construct. These changes were the same as those made in the mutant probe that eliminated nuclear protein binding. When transfected into BAEC, the mutant construct was expressed at 2–10-fold higher levels than those of the wild-type sequence (Fig. 6F and data not shown). These data suggest that the proximal Ets elements bind to one or more members of the Ets family, including GABP.

To determine the potential function of the murine proximal Ets element, we changed the proximal Ets sequence AGGAAG to AGCTAG in the mp1379LUC construct. These changes were the same as those made in the mutant probe that eliminated nuclear protein binding. When transfected into BAEC, the mutant construct was expressed at 2–10-fold higher levels than those of the wild-type sequence (Fig. 6F and data not shown). These data suggest that the proximal Ets elements play a negative regulatory role in transcription of the murine P-selectin gene.

**TNF-α or LPS Increases Luciferase Reporter Gene Expression Driven by the Murine but Not the Human P-selectin 5′-Flanking Region**—To test whether differences in the promoters of human and murine P-selectin gene accounted for the differential regulatory sequences are underlined. The species-specific putative regulatory elements are underlined and italicized. The major transcription start site at position 134 is in bold.
response of P-selectin to TNF-α or LPS, we compared the abilities of TNF-α or LPS to induce expression of a reporter gene driven by the murine or human P-selectin 5'-flanking region. Following transfection of the constructs into BAEC, the cells were treated with fresh medium in the presence or absence of LPS or TNF-α for various intervals, and the cells were then harvested for luciferase activity assays. As shown in Fig. 7, the reporter gene driven by the 5'-flanking region of the murine P-selectin gene from −1379 to −13 promoted constitutive expression of luciferase that was further increased 5–15-fold after the transfected cells were stimulated with TNF-α or LPS for 4.5 h. In sharp contrast, the reporter gene driven by the 5'-flanking region of the human P-selectin gene from −1339 to −13 promoted constitutive expression that was not further increased after the same treatment. Similar results were obtained when the transfected cells were stimulated with TNF-α or LPS for 6 or 8 h. The failure to induce expression of the human P-selectin reporter gene was not due to excessive basal expression, because the levels of constitutive expression were similar for both the murine and human P-selectin reporter genes (data not shown). These data demonstrate that the 5'-flanking regions of both the murine and human P-selectin genes confer constitutive expression in endothelial cells. However, only the murine sequence has elements that allow inducible expression in response to TNF-α or LPS.

Deletional Analysis of the Murine P-selectin 5'-Flanking Region—To localize regulatory elements required for the constitutive or inducible expression of the murine P-selectin gene, we prepared reporter constructs in which serially truncated fragments of the 5'-flanking region were linked to luciferase. Following transfection of the constructs into BAEC, the cells were incubated with or without TNF-α or LPS and then assayed for luciferase activity. As shown in Fig. 8, deletion to position −593 decreased constitutive expression of the luciferase reporter gene by ~50% but did not affect the degree of induction by LPS or TNF-α. Deletion to −474 did not further decrease constitutive expression but did significantly reduce inducible expression. Deletion to −349 slightly increased constitutive expression but did not further affect inducible expression. Deletions to −288, −229, −164 or −148 only modestly affected constitutive or inducible expression. Deletions to −110 or −91 drastically reduced both constitutive and inducible expression. These data suggest that the sequence from −1379 to −593 contains a positive regulatory element(s) for constitutive expression, and the sequence from −593 to −474 contains a regulatory element(s) for TNF-α- or LPS-mediated inducible expression. Sequences proximal to −229 appear to be required for both constitutive and inducible expression.

Fig. 4. Nuclear protein binding activity and function of the conserved GATA element. A, upper strand sequences of the oligonucleotides used as probes and competitors in the gel mobility shift assay. The murine P-selectin GATA/TATA element is from the indicated region of the 5'-flanking region of the murine P-selectin gene. The mutant oligonucleotide contains three nucleotide changes in the core GATA sequence. The human P-selectin and endothelin-1 GATA probes were characterized previously (25, 53, 54). B, competitive gel mobility shift assay. Nuclear extracts from HEL cells were incubated with a 32P-labeled endothelin-1 GATA element in the presence of a 100-fold molar excess of the indicated unlabeled competitor. C, the same mutations of the GATA/TATA element were introduced into a luciferase reporter gene driven by the murine P-selectin 5'-flanking region sequence from −1379 to −13. Luciferase activity was measured following transfection of the wild-type and mutant reporter genes into BAEC. The luciferase activity of the wild-type reporter gene was normalized to 100%. The data represent the mean ± S.D. of three experiments. Three independent transfections were performed in each experiment.
We cloned the 5′-flanking region of the murine gene and compared its features with those previously reported for the human gene. Both genes shared several conserved regulatory elements, including functional GATA and Ets elements. However, several unique aspects of the murine gene were revealed, including several elements that may explain why TNF-α or LPS augments transcription of the P-selectin gene in murine but not in human endothelial cells.

The human P-selectin promoter initiates transcription at multiple sites (25). In contrast, the murine promoter initiates transcription predominantly at a single site. Both genes have functional proximal GATA sites. However, unlike the human GATA site, the murine site deviates one nucleotide from the consensus GATA sequence, leading to weaker binding to GATA proteins. Furthermore, the murine GATA site overlaps a weak TATA box located 21 bp upstream of the transcription start site. We refer to the murine GATA site as a GATA/TATA element, because it resembles the GATA/TATA elements in the rat platelet factor-4 and chicken β-globin genes that can bind to either TFIID or GATA proteins (28, 57, 58). This dual-binding property may allow the GATA/TATA element to communicate with a distal tissue-specific enhancer. In the chicken β-globin gene, the protein GATA-1, when bound to a proximal GATA/TATA element, may interact with GATA-1 or other proteins bound to a distal 3′ enhancer that contains both GATA ele-

**FIG. 5.** Nuclear protein binding activities of the murine P-selectin tandem GATA and AP-1-like elements. A, sequence comparison of the enhancers in the hypersensitive sites 2 or 3 (HS2 or HS3) of the murine β-globin locus control region (46) and in the murine P-selectin insertion. Also indicated are the upper strand sequences of the wild-type and mutant oligonucleotides encoding the murine P-selectin tandem GATA elements or AP-1-like elements used in gel mobility shift assays. B, nuclear extracts from bEnd.3 cells were incubated with labeled wild-type or mutant (Mut.) P-selectin tandem GATA probes in the absence or the presence of the indicated unlabeled competitor. C, nuclear extracts from HEL cells were incubated with the labeled tandem GATA probe in the absence or the presence of the indicated antibody. D, nuclear extracts from bEnd.3 cells were incubated with the tandem AP-1-like probe in the absence or the presence of the indicated unlabeled competitor.
Promoters of Murine and Human P-selectin Genes

10065

FIG. 6. Nuclear protein binding activity and function of the proximal Ets element. A, upper strand sequences of the oligonucleotides encompassing the proximal Ets elements from the 5′-flanking elements of the human and murine P-selectin genes. The mutant oligonucleotides have two nucleotide changes (AGGAA to AGCTA) in the core region of the human or murine Ets element. The oligonucleotide encoding a GABP recognition site was derived from the HSV1 IE gene (56). B, the labeled murine proximal Ets oligonucleotide was incubated with BAEC nuclear extracts in the absence or presence of the indicated competitor. The experiments were transfected with a reporter gene driven by the murine, but not in human endothelial cells. TNF-α or LPS increased expression of a reporter gene driven by the murine, but not the human, P-selectin promoter in transfected BAEC. Serial deletions of the murine 5′-flanking region identified two segments that were required for the TNF-α- or LPS-mediated induction of transcription. The first segment from −593 to −474 contained two tandem putative kB sites, whereas the second segment proximal to −229 contained a reverse-oriented kB site and a variant activating transcription factor/cAMP sequence. In the accompanying paper (68), we demonstrate that all these elements are required for TNF-α or LPS to

FIG. 7. The 5′-flanking region of the murine but not the human P-selectin gene confers TNF-α- or LPS-mediated transcriptional induction of a reporter gene in transfected BAEC. BAEC were transfected with a reporter gene driven by either the −1379 to −13 sequence of the murine P-selectin gene or the −1339 to −13 sequence of the human P-selectin gene. After 40 h, the transfected cells were then harvested for assay of luciferase activity.

elements and NF-E2 (AP1-like) elements (58). This interaction allows the two control regions to form a DNA loop. Once the DNA loop is formed, TFIID and adapter proteins displace GATA-1 and produce an active initiation complex under control of the erythroid-specific enhancer. Intriguingly, the murine P-selectin promoter contains an upstream region composed of tandem GATA elements and tandem AP-1-like elements. Furthermore, the murine P-selectin tandem GATA elements bound to at least one GATA protein (GATA-2), and the tandem AP-1-like elements bound to a still uncharacterized nuclear protein. Deletion of the sequence from −1379 to −593, which contains these elements, decreased expression of a luciferase reporter gene in BAEC by 50%. These data suggest that the murine GATA/TATA element may cooperate with this putative enhancer, in a manner much like that proposed for the β-globin gene. The murine putative enhancer is located in an insertion that is not present in the corresponding portion of the human P-selectin gene. It is not known whether the human gene has a similar enhancer in a different location.

Ets elements, which are often associated with GATA elements, are in the promoters of many genes whose expression is restricted to hematopoietic and/or endothelial cells (25, 27–34). The functions of most Ets elements have not been well characterized, although many Ets proteins are known transcriptional activators. Many Ets elements are adjacent to other regulatory elements, which refine their binding specificities for particular Ets proteins (43). Two conserved Ets elements were identified in both the mouse and human P-selectin genes. Interestingly, the distances between the two murine Ets elements (224 bp) and the two human elements (213 bp) differ by exactly one turn of the DNA helix. The distal Ets element is adjacent to a conserved AT-rich HOX recognition sequence (41), and the proximal Ets element is flanked by conserved 4-bp A/T sequences. Mutation of the proximal Ets element eliminated binding to an Ets protein, most likely the ubiquitously expressed GABP, and increased reporter gene expression directed by the murine P-selectin promoter by 2–10-fold in transfected BAEC. The α-subunit of GABP (GABPα) shares sequence similarity with the Ets family of transcription factors, and the β-subunit contains four ankyrin repeats that mediate the interaction with GABPα (56). GABPβ dimerizes to form an α2β2 tetramer that binds with high affinity to two Ets elements separated by variable distances (56, 59). Although the two conserved Ets elements in the P-selectin genes are separated by −200 bp in linear sequence, a nucleosome (60, 61) could bring the two elements sufficiently close to interact simultaneously with a GABP tetramer to form a DNA loop. By a similar mechanism, the three DNA-binding domains of the architectural HMG I(Y) protein may interact with the three A/T-rich sequences adjacent to the Ets elements to stabilize the loop (62). Formation of this putative loop may be regulated. For example, oxidation of GABPα destroys its DNA binding activity (63), a Hox protein may bind to the conserved 4-bp AT sequence adjacent to the distal Ets element (41), and interleukin-4-dependent signaling may modulate the DNA binding of HMG I(Y) through phosphorylation (64). Mutation of the murine proximal Ets element increased reporter gene expression. This suggests that the putative Ets-mediated DNA looping may repress transcription. Transcriptional repression mediated by DNA looping has been observed in other genes (65). Although deletion of the distal Ets element did not alter reporter gene expression, large deletions may alter other regulatory elements. Furthermore, data from transient transfection of reporter genes may not fully reflect the architecture and function of genes in the intact chromosome (66, 67).

Our findings also suggest a molecular explanation for the ability of TNF-α or LPS to augment expression of P-selectin in murine but not in human endothelial cells. TNF-α or LPS increased expression of a reporter gene driven by the murine, but not the human, P-selectin promoter in transfected BAEC. Serial deletions of the murine 5′-flanking region identified two segments that were required for the TNF-α- or LPS-mediated induction of transcription. The first segment from −593 to −474 contained two tandem putative kB sites, whereas the second segment proximal to −229 contained a reverse-oriented kB site and a variant activating transcription factor/cAMP sequence. In the accompanying paper (68), we demonstrate that all these elements are required for TNF-α or LPS to
maximally induce transcription of the murine P-selectin gene. These sites are not present in the 5′-flanking region of the human P-selectin gene. Instead, the human gene has a novel κB site that binds homodimers of p50 or p52 but not homodimers or heterodimers containing p65 (26). Thus, the differential ability of TNF-α or LPS to induce expression of P-selectin in mice, but not in humans, may result in part from differences in the promoters of the respective genes.

The species-specific transcriptional regulation of P-selectin in response to TNF-α or LPS is both unusual and surprising, given the apparent similarities of many selective functions across species (69–71). However, there are other known genes in which species-specific changes in regulatory elements affect responsiveness to particular mediators (72, 73). Although the biological implications of these species-specific regulatory mechanisms remain unexplained, the presence of such mechanisms suggests caution in extrapolating the results of some inducible gene responses in mouse models to humans.

Acknowledgments—We thank Ginger Hampton for technical assistance. We are grateful to Dr. James Morrissey for critical reading of the manuscript. We also thank Dr. Kenneth Jackson (Molecular Biology Resource Facility, University of Oklahoma Health Sciences Center) for synthesis of oligonucleotides.

REFERENCES

1. Springer, T. A. (1994) Cell 76, 301–314
2. Collins, T., Read, M. A., Neish, A. S., Whitley, M. Z., Thanos, D., and Maniatis, T. (1995) FASEB J. 9, 899–909
3. McEver, R. P. (1997) in The Selectins: Initiators of Leukocyte Endothelial Adhesion (Vesell, D., ed) pp. 31–47, Harwood Academic Publishers, Amsterdam
4. Bevilacqua, M. P., Stengelin, S., Gimbrone, M. A., Jr., and Seed, B. (1989) Science 243, 1160–1165
5. MotLEY, K. F., Osborn, L., Hessien, C., Tizard, R., Goff, D., Vassallo, C., Tarr, P. J., Bonnycastle, R., Lobb, R., Harlan, J. M., and Pohlan, T. H. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 6523–6527
6. Leedham, H. C., and Parks, T. P. (1995) J. Biol. Chem. 270, 933–943
7. Lenzoeramo, M. F., McQuillan, J. J., Rosen, G. D., and Dean, D. C. (1992) J. Biol. Chem. 267, 16329–16332
8. Neish, A. S., Read, M. A., Thanos, D., Fine, R., Maniatis, T., and Collins, T. (1995) Mol. Cell. Biol. 15, 2553–2559
9. Takeuchi, M., and Baichwal, V. R. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3561–3565
10. Stenberg, P. E., McEver, R. P., Shuman, M. A., Jacques, Y. V., and Bainton, D. F. (1985) J. Cell Biol. 101, 880–886
11. Berman, C. L., Yeo, E. L., Wencel-Drake, J. D., Furie, B. C., Ginsberg, M. H., and Furie, B. (1986) J. Clin. Invest. 78, 130–137
12. McEver, R. P., Beckstead, J. H., Moore, K. L., Marshall-Carlson, L., and Bainton, D. F. (1989) J. Clin. Invest. 84, 92–99
13. Bonfanti, R., Furie, B. C., Furie, B., and Wagner, D. D. (1989) Blood 73, 1109–1112
14. Disseld, M., Morrissey, J. H., Fugate, R. D., Bainton, D. F., and McEver, R. P. (1992) Mol. Biol. Cell 3, 309–321
15. Hattori, R., Hamilton, K. K., Fugate, R. D., McEver, R. P., and Sims, P. J. (1989) J. Biol. Chem. 264, 7768–7771
16. Larsen, E., Cel, A., Gilbert, G. E., Furie, B. C., Erban, J. K., Bonfanti, R., Wagner, D. D., and Furie, B. (1989) Cell 59, 305–312
17. Geng, J.-G., Bevilacqua, M. P., Moore, K. L., McIntyre, T. M., Prescott, S. M., Kim, J. M., Bliss, G. A., Zimmerman, G. A., and McEver, R. P. (1990) Nature 343, 757–760
18. Hamburger, S. A., and McEver, R. P. (1990) Blood 75, 550–554
19. Lawrence, M. B., and Springer, T. A. (1991) Cell 65, 859–873
20. Weller, A., Isenmann, S., and Vestweber, D. (1992) J. Biol. Chem. 267, 15176–15183
21. Sanders, W. E., Wilson, R. W., Ballantyne, C. M., and Beaudet, A. L. (1992) Blood 80, 795–809
22. Yao, L., Pan, J., Setiadi, H., Patel, K. D., and McEver, R. P. (1996) J. Exp. Med. 184, 81–92
23. Hahne, M., Jager, U., Isenmann, S., Hallmann, R., and Vestweber, D. (1993) J. Cell Biol. 121, 655–664
24. Burns, S. A., DeGuzman, B. J., Newburger, J. W., Mayer, J. E., Jr., Neufeld, E. J., and Briscoe, D. M. (1995) J. Thromb. Haemost. 110, 924–933
25. Pan, J., and McEver, R. P. (1993) J. Biol. Chem. 268, 22600–22608
26. Pan, J., and McEver, R. P. (1995) J. Biol. Chem. 270, 23077–23083
27. Uzun, G., Prenant, M., Prandini, M.-H., Martin, F., and Marguerie, G. (1991) J. Biol. Chem. 266, 8925–8930
28. Ravid, K., Doh, T., Beeler, D. L., Kuter, D. J., and Rosenberg, R. D. (1991) Mol. Cell. Biol. 11, 6116–6127
29. Hickey, M. J., and Roth, G. J. (1993) J. Biol. Chem. 268, 4343–4344
30. Jhahari, R., and Lynch, D. C. (1994) Mol. Cell. Biol. 14, 999–1008
31. Hashimoto, Y., and Waje, J. (1995) J. Biol. Chem. 270, 24532–24538
32. Schlaefer, T. M., Bartunkova, S., Lawitts, J. A., Teichmann, G., Risau, W., Deutsch, U., and Sato, T. N. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 3058–3063
33. Morishita, K., Johnson, D. E., and Williams, L. T. (1995) J. Biol. Chem. 270, 27948–27953
34. Patterson, C., Perrella, M. A., Hess, C. M., Yoshizumi, M., Lee, M. E., and Haber, E. (1996) J. Biol. Chem. 271, 31111–31118
35. Johnston, G. I., Bliss, G. A., Newman, P. J., and McEver, R. P. (1990) J. Biol. Chem. 265, 21381–21385
36. Johnston, G. I., Cook, R. G., and McEver, R. P. (1989) Cell 56, 1033–1044
37. DeWet, J. R., Wood, K. V., DeLuca, M., Helinski, D. R., and Subramani, S. (1989) Mol. Cell. Biol. 9, 6419
38. Schreiber, E., Matthias, P., Muller, M. M., and Schaffner, W. (1989) Nucleic Acids Res. 17, 6419
39. Dignam, J. D., Lebowitz, R. M., and Roeder, R. (1983) Nucleic Acids Res. 11, 1475–1499
40. Koop, B. F., and Hord, L. (1994) Nat. Genet. 7, 48–53
41. Catron, K. M., Iler, N., and Abate, C. (1993) Mol. Biol. Cell. 4, 1160–1165
42. Law, B. F., and Hood, L. (1994) Science 265, 6662–6665
43. Wasylyk, B., Hahn, S. L., and Giovane, A. (1993) Eur. J. Biochem. 211, 7–18
44. Burglin, T. R. (1991) Cell 66, 11–12
45. Solomon, M. J., Strauss, F., and Varshavsky, A. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 1276–1280
46. Stamatoyannopoulos, J. A., Goodwin, A., Joyce, T., and Lowrey, C. H. (1995) *EMBO J.* 14, 106–116
47. Kunsch, C., Ruben, S. M., and Rosen, C. A. (1992) *Mol. Cell. Biol.* 12, 4412–4421
48. Angel, P., and Karin, M. (1991) *Biochem. Biophys. Acta* 1072, 129–157
49. Read, M. A., Whitley, M. Z., Gupta, S., Pierce, J. W., Best, J., Davis, R. J., and Collins, T. (1997) *J. Biol. Chem.* 272, 2753–2761
50. Whitley, M. Z., Thanos, D., Read, M. A., Maniatis, T., and Collins, T. (1994) *Mol. Cell. Biol.* 14, 6444–6475
51. Schindler, U., and Baichwal, V. R. (1994) *Mol. Cell. Biol.* 14, 5820–5831
52. Meacock, S., Pescini-Gobert, R., DeLamarter, J. F., and van Huijsduijnen, R. H. (1994) *J. Biol. Chem.* 269, 31756–31762
53. Lee, M.-E., Bloch, K. D., Clifford, J. A., and Quertermous, T. (1990) *Science* 253, 762–768
54. Dorfman, D. M., Wilson, D. B., Bruns, G. A., and Orkin, S. H. (1992) *J. Biol. Chem.* 267, 1277–1285
55. Baldwin, A. S., Jr., and Sharp, P. A. (1988) *Proc. Natl. Acad. Sci. U. S. A.* 85, 723–727
56. Thompson, C. C., Brown, T. A., and McKnight, S. L. (1991) *Science* 253, 762–768
57. Aird, W. C., Parvin, J. D., Sharp, P. A., and Rosenberg, R. D. (1994) *J. Biol. Chem.* 269, 883–889
58. Fong, T. C., and Emerson, B. M. (1992) *Genes Dev.* 6, 521–532
59. Virbasius, J. V., Virbasius, C.-M. A., and Scarpulla, R. C. (1993) *Genes Dev.* 7, 380–392
60. Wolfe, A. P. (1994) *Science* 264, 1100–1101
61. Workman, J. L., and Buchman, A. R. (1993) *Trends Biochem. Sci.* 18, 90–95
62. Maher, J. F., and Nathans, D. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 6716–6720
63. Martin, M. E., Chinénov, Y., Yu, M., Schmidt, T. K., and Yang, X. Y. (1996) *J. Biol. Chem.* 271, 25617–25623
64. Wang, D. Z., Ray, P., and Boothby, M. (1995) *J. Biol. Chem.* 270, 22924–22932
65. Zwieb, C., Kim, J., and Adhya, S. (1997) *Genes Dev.* 3, 606–611
66. Archer, T. K., Leffebvre, P., Wolford, R. G., and Hager, G. L. (1992) *Science* 255, 1573–1576
67. Smith, C. L., and Hager, G. L. (1997) *J. Biol. Chem.* 272, 27493–27496
68. Pan, J., Xia, L., Yao, L., and McEver, R. P. (1998) *J. Biol. Chem.* 273, 10068–10077
69. McEver, R. P., Moore, K. L., and Cummings, R. D. (1995) *J. Biol. Chem.* 270, 11025–11030
70. Kansas, G. S. (1996) *Blood* 88, 3259–3287
71. Frenette, P. S., and Wagner, D. D. (1997) *Thromb. Haemostasis* 78, 60–64
72. Pajovic, S., Jones, V. E., Proos, K. R., Berger, P. G., and Baumann, H. (1994) *J. Biol. Chem.* 269, 2215–2224
73. Holmberg, M., Leonardsson, G., and Ny, T. (1995) *Eur. J. Biochem.* 231, 466–474
Comparison of Promoters for the Murine and Human P-selectin Genes Suggests Species-specific and Conserved Mechanisms for Transcriptional Regulation in Endothelial Cells

Junliang Pan, Lijun Xia and Rodger P. McEver

J. Biol. Chem. 1998, 273:10058-10067.
doi: 10.1074/jbc.273.16.10058

Access the most updated version of this article at http://www.jbc.org/content/273/16/10058

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 72 references, 53 of which can be accessed free at http://www.jbc.org/content/273/16/10058.full.html#ref-list-1