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The c-*myc*-Regulated Gene *mrl* Encodes Plasminogen Activator Inhibitor 1

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The DNA sequence of the c-*myc*-regulated gene *mrl* (G. C. Prendergast and M. D. Cole, Mol. Cell. Biol. 9:124–134, 1989) reveals that it encodes plasminogen activator inhibitor 1 (PAI-1), a regulator of extracellular proteolysis. Comparison of the human and mouse PAI-1 promoters and cDNA 3′ noncoding regions revealed several highly conserved sequence domains, potential targets for c-*myc* and other factors influencing PAI-1 expression. We discuss possible roles for PAI-1 in normal and neoplastic cell growth control.

Identification of the targets of oncogene activity is a central problem in the molecular biology of cancer. The c-*myc* oncogene encodes a nuclear protein which plays an important role in normal and abnormal cell proliferation (reviewed in references 7 and 14), although its targets and molecular function remain unknown. We recently identified a novel serum-regulated cellular gene, *mrl*, which is deregulated in c-*myc*-immortalized primary rodent fibroblasts and specifically induced by c-*myc* protein in 3T3 cell lines (30). The DNA sequence of murine *mrl* indicates that it is the rodent homolog of plasminogen activator inhibitor 1 (PAI-1), a regulator of extracellular proteases involved with various normal and pathological phenomena, including neoplasia (reviewed in references 8, 15, and 32).

A full-length murine *mrl* cDNA was obtained by standard methods (23) from a cDNA library constructed with RNA from BALB/c 3T3 cells treated with fetal calf serum plus cycloheximide (17), conditions which strongly induce *mrl* expression (G. C. Prendergast, Ph.D. thesis, Princeton University, Princeton, N.J., 1989). A restriction map and sequencing strategy are shown in Fig. 1A, and the complete cDNA sequence and predicted translation product are shown in Fig. 1B. The *mrl* cDNA is 3,014 base pairs (bp) long and contains a 1,206-bp open reading frame, encoding a 402-amino-acid polypeptide with a calculated molecular mass of 45,167 daltons. The predicted polypeptide sequence is ~78% identical (~82% with conservative substitutions) to that encoded by human PAI-1 cDNA (1, 26, 28) (Fig. 1C). A comparison of the 3′ noncoding domains in the human and mouse PAI-1 cDNAs revealed extensive similarity in a 200-bp region about 400 bp upstream of the poly(A) tract (Fig. 2). Within this region is an unusual 100-bp stretch, starting at nucleotide 2445 in the mouse cDNA, which is >90% identical to the human PAI-1 message. The high conservation strongly suggests a role for this element in regulation of PAI-1 gene expression.

Transcriptional induction of PAI-1 in 3T3 cells by platelet-derived growth factor (30) prompted us to locate control elements in the PAI-1 promoter. As a first step toward this goal, we searched the mouse PAI-1 promoter for DNA sequence conserved in the human gene (3). Genomic clones were isolated from an EMBL 3 library (Clontech Laboratories), and a region of ~1.4 kilobases encompassing the murine promoter and noncoding exon 1 was sequenced and compared with the human gene (Fig. 3). Three broad segments of conserved DNA sequence were observed. The first was across the exon 1 splice donor junction, a 45-bp block which is 87% identical to human PAI-1. The second was an ~210-bp segment within the proximal region of the promoter. At 29 bp upstream of the 5′ PAI-1 mRNA cap site was a TATAA transcription start consensus sequence, which was embedded within a 65-bp region with 96% identity between human and mouse genes (proximal box). Two cap sites are utilized by the PAI-1 promoter in mouse 3T3 fibroblasts, both adenosine residues; each site is used with about equal efficiency (Prendergast, Ph.D. thesis; data not shown). Approximately 400 bp distal to the TATAA consensus is a 300-bp segment which is 76% identical between the genes; within this region are several blocks of sequence identity (distal box). Relative to the human PAI-1 promoter, the mouse promoter has a gap of ~100 bp between the proximal and distal boxes of homology.

As the first cellular gene known to be regulated by c-*myc* protein in stable cell assays (30), the PAI-1 gene may be potentially useful as a substrate with which to uncover the molecular function of the c-*myc* oncoprotein. PAI-1 is somewhat unusual (like c-*myc*) in that it is expressed during both the G_0 to G_1 cell cycle transition and in cycling cells (30), which do not enter the G_0 phase (29). More commonly, serum- and platelet-derived growth factor-regulated genes are well expressed during G_0 to G_1 but not in cycling cells (31; unpublished observations). We speculate that PAI-1 expression is required generally during fibroblast proliferation. Platelet-derived growth factor is added to a growing list of factors which regulate the PAI-1 gene in several systems, including transforming growth factor-beta (16, 21), glucocorticoids (12), interleukin-1 (2), lipopolysaccharide (33), tumor necrosis factor alpha (33), thrombin (10) and other factors (see reference 33 for an extensive listing). PAI-1 cDNA has also been isolated by Bravo and his associates (4) in a screen for serum-induced mRNAs in NIH 3T3 cells.

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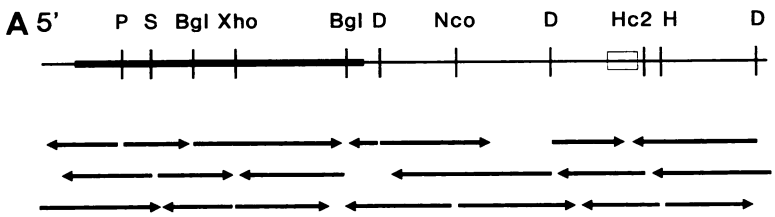

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GCCAATCACAAGGCACCTTTGAATACTCAGGATGCAGATGTCTTCAGCCCTTGCTTGCCTCATCCTGGGCCTGGTTCTGGTCTCTGGGAAAGGGTTCACT 200
24  L  P  L  R  E  S  H  T  A  H  Q  A  T  D  F  G  V  K  V  F  Q  Q  V  V  Q  A  S  K  D  R  N  V  V  F
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58    S  P  Y  G  V  S  S  V  L  A  M  L  Q  M  T  T  A  G  K  T  R  R  Q  I  Q  D  A  M  G  F  K  V  N
TCTCTCCCTATGGCGTGTCCTCGGTGCTGGCTATGCTGCAGATGACCACAGCGGGGAAAACCCGGCGGCAGATCCAAGATGCTATGGGATTCAAAGTCAA 400
91    E  K  G  T  A  H  A  L  R  Q  L  S  K  E  L  M  G  P  W  N  K  N  E  I  S  T  A  D  A  I  F  V  Q
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124 R  D  L  E  L  V  Q  G  F  M  P  H  F  F  K  L  F  Q  T  M  V  K  Q  V  D  F  S  E  V  E  R  A  R  F
CGGGACCTAGAGCTGGTCCAGGGCTTCATGCCCCACTTCTTCAAGCTCTTCCAGACTATGGTGAAACAGGTGGACTTCTCAGAGGTGGAAAGAGCCAGAT 600
158   I  I  N  D  W  V  E  R  H  T  K  G  M  I  N  D  L  L  A  K  G  A  V  D  E  L  T  R  L  V  L  V  N
TTATCATCAATGACTGGGTGGAAAGGCATACCAAAGGTATGATCAATGACTTACTGGCCAAGGGGGCTGTAGACGAGCTGACACGCCTGGTGCTGGTGAA 700
191   A  L  Y  F  S  G  Q  W  K  T  P  F  L  E  A  S  T  H  Q  R  L  F  H  K  S  D  G  S  T  V  S  V  P
TGCCCTCTACTTCAGTGGCCAATGGAAGACCCCTTTCTTAGAGGCCAGCACCCACCAGCGCCTCTTCCACAAGTCTGATGGCAGCACCGTCTCTGTGCCC 800
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ATGATGGCTCAGAGCAACAAGTTCAACTACACTGAGTTCACCACCCCCGATGGGCTCGAGTATGACGTCGTGGAACTGCCCTACCAGCGGGACACCCTCA 900
258   M  F  I  A  A  P  F  E  K  D  V  H  L  S  A  L  T  N  I  L  D  A  E  L  I  R  Q  W  K  G  N  M  T
GCATGTTCATCGCTGCACCCTTTGAGAAAGATGTGCACCTCTCCGCCCTCACCAACATCTTGGATGCTGAACTCATCAGACAATGGAAGGGCAACATGAC 1000
291   R  L  P  R  L  L  I  L  P  K  F  S  L  E  T  E  V  D  L  R  G  P  L  E  K  L  G  M  P  D  M  F  S
CAGGCTGCCCCGCCTCCTCATCCTGCCTAAGTTCTCTCTGGAGACTGAAGTGGACCTCAGAGGGCCCCTGGAGAAGTTGGGCATGCCTGACATGTTTAGT 1100
324 A  T  L  A  D  F  T  S  L  S  D  Q  E  Q  L  S  V  A  Q  A  L  Q  K  V  R  I  E  V  N  E  S  G  T  V
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358   A  S  S  S  T  A  F  V  I  S  A  R  M  A  P  T  E  M  V  I  D  R  S  F  L  F  V  V  R  H  N  P  T
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391   E  T  I  L  F  M  G  Q  V  M  E  P  *
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AAAAAAAAAAAAA 3014
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FIG. 1. *mrl* cDNA encodes murine PAI-1. (A) Restriction map and sequencing strategy of a full-length murine *mrl* cDNA. The dark bar represents the 1,206-bp polypeptide open reading frame; the open box indicates a 3′ noncoding region highly similar to the human PAI-1 cDNA (see text). Enzyme abbreviations are BgI, *Bgl*I; D, *Dra*I; H, *Hin*dIII; Hc2, *Hin*dII; Nco, *Nco*I; S, *Sac*I; Xho, *Xho*I. (B) Complete DNA sequence of the *mrl* cDNA. The single-letter amino acid code is used for the inferred translation product of the 1,206-bp polypeptide open reading frame. (C) Amino acid similarity between human PAI-1 and the inferred polypeptide encoded by the *mrl* cDNA. Dashes indicate identity; colons indicate conservative substitutions.

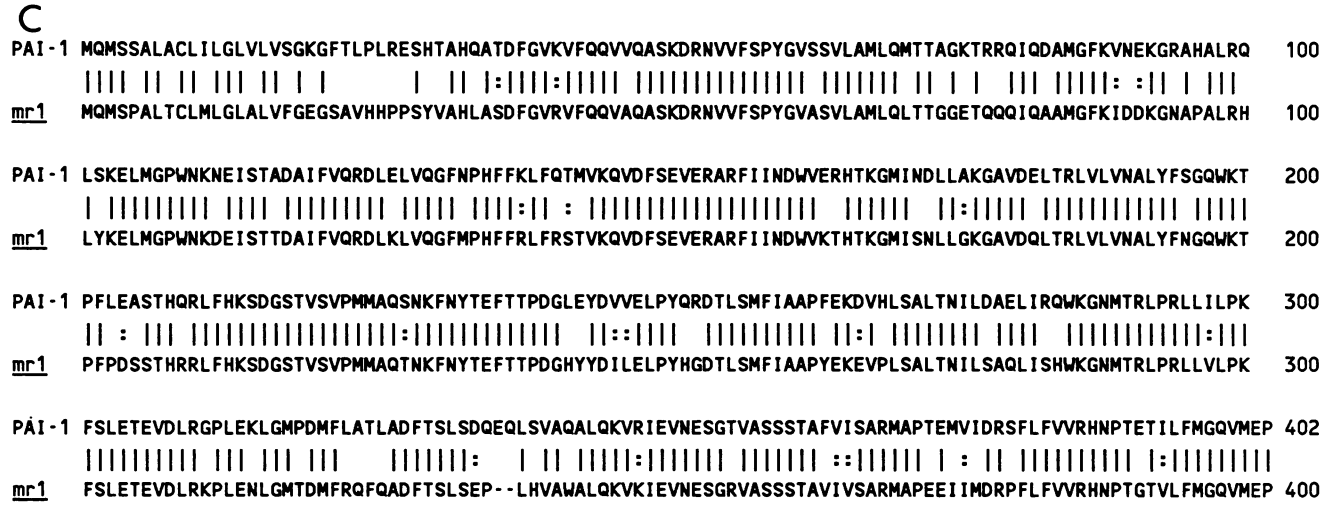

FIG. 1.—*Continued.*

Since it is targeted by c-*myc* and other factors which influence cell cycle decisions in fibroblasts, PAI-1 may play a role in normal and neoplastic cell growth control. However, it is not immediately clear how its protease inhibition activity might fit into the picture. Along with two other well-characterized PAIs, PAI-2 and protease nexin, PAI-1 is a member of the serine protease inhibitor superfamily of proteins (serpins [5]). PAIs control a variety of physiological processes including matrix turnover, cell migration, coagulation, fibrinolysis, complement activation, and inflammatory reactions (reviewed in references 15, 32, and 34). Early studies of PAI-1 centered on the characterization of its ability to rapidly inhibit the proteinase activity of tissue-type PA, a central regulator of the fibrinolytic cascade (9, 19, 20, 35). The finding that PAI-1 localizes in the extracellular matrix of tissue culture cells (16, 18) and interacts specifically with vitronectin (36) is intriguing in light of the role of extracellular proteolytic enzymes in tissue remodeling, tumor invasion, and metastasis (8, 25, 27). Potential roles for PAIs in affecting cell growth indirectly, for example, via extracellular matrix organization, have been discussed in recent reviews (15, 32). The finding that c-*myc* induces expression of a PAI might be considered somewhat counterintuitive, given that activation (rather than suppression) of protease activity is known to be important in tumorigenesis. Consistent with the fact that a protease inhibitor is targeted by *myc* is the observation that c-*myc*-immortalized primary fibroblasts (which exhibit deregulated PAI-1 expression) are neither morphologically transformed nor tumorigenic (13). Others have reported that rat-1 cells morphologically transformed by v-*myc* have decreased steady-state PAI-1 protein levels compared with parental rat-1 cells (6), although the level of PAI-1 transcription or mRNA was not examined. However, even if these latter levels of PAI-1 gene expression were down-regulated, it is likely that the cellular context (e.g., flat immortalized versus morphologically transformed cells) would impinge on the regulation of PAI-1 by c-*myc*.

There exists some circumstantial support in the literature for the proposition that extracellular protease inhibitors can directly influence mitogenic decisions. First, evidence that plasmin can activate the latent serum form of transforming growth factor-beta in vitro (22) suggests a role for PAI-1 in regulating the cellular response to this factor. Second, two growth factors secreted by human HepG2 hepatoma cells (endothelial cell growth factors 2a and 2b) which stimulate the growth of human endothelial cells in serum-free monolayer culture have been found to be protease inhibitors (24). Finally, the glia-derived neurite-promoting factor, a serine protease inhibitor, is reported to be mitogenic for astrocytic cells (11). One might speculate that the serine protease inhibition activity of PAI-1 is not relevant to normal or transformed cell growth. This notion is intriguing because it implies that PAI-1 has other undefined functions in addition

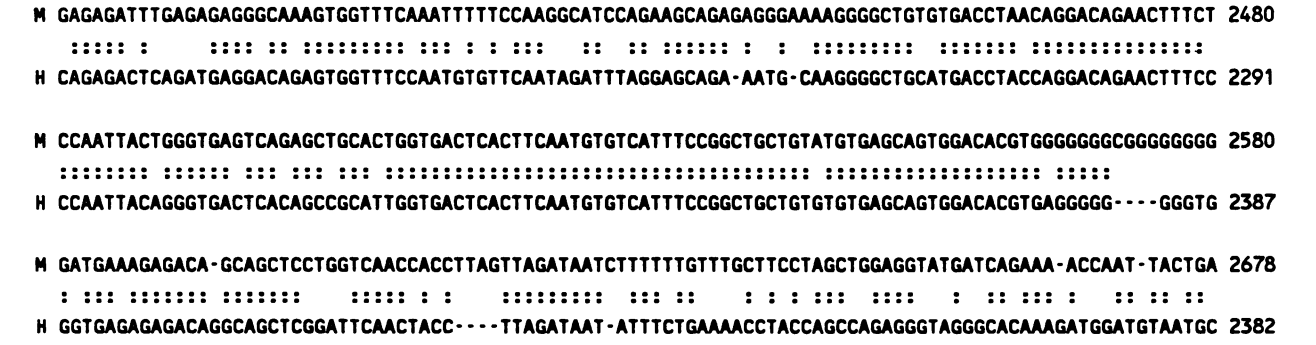

FIG. 2. Sequence similarity between 3′ noncoding regions of murine and human PAI-1 genes. The human sequence is taken from Ny et al. (26).

FIG. 3. Sequence similarity between 5′ noncoding regions of the murine and human PAI-1 genes. The human sequence and mRNA cap designation are from Bosma et al. (3). The asterisks designate exon 1, and the carats indicate the mRNA caps sites; the TATAA consensus is underlined.

to its well-characterized role in protease inhibition. Clearly, further studies are necessary to assess the contributions of PAI-1 to cell growth decisions.

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