

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

GEORGE C. PRENDERGAST,*† LISA E. DIAMOND, DOUGLAS DAHL,
AND MICHAEL D. COLE

Department of Biology, Lewis Thomas Laboratory, Princeton University,
Princeton, New Jersey 08544-1014

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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 G₀ to G₁ cell cycle transition and in cycling cells (30), which do not enter the G₀ phase (29). More commonly, serum- and platelet-derived growth factor-regulated genes are well expressed during G₀ to G₁ 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.

* Corresponding author.

† Present address: Department of Biochemistry, New York University Medical Center, 550 First Avenue, New York, NY 10016.

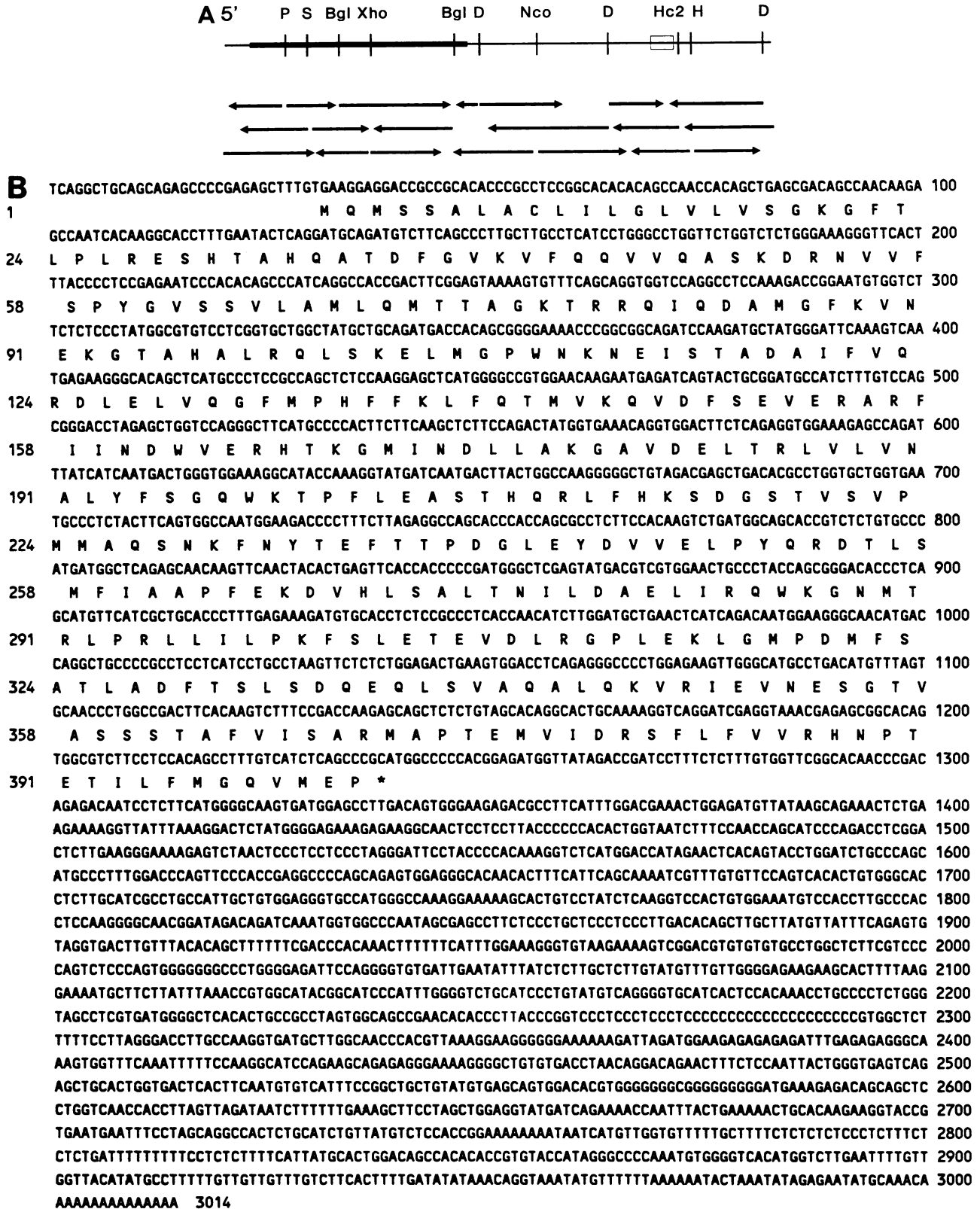


FIG. 1. *mrl* cDNA encodes murine PAI-1. (A) Restriction map and sequencing strategy of a full-length murine *mrl* cDNA. The dark bar represents the 1,206-bp polypeptide open reading frame; the open box indicates a 3' noncoding region highly similar to the human PAI-1 cDNA (see text). Enzyme abbreviations are Bgl, *Bgl*II; D, *Dra*I; H, *Hind*III; Hc2, *Hind*II; 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.

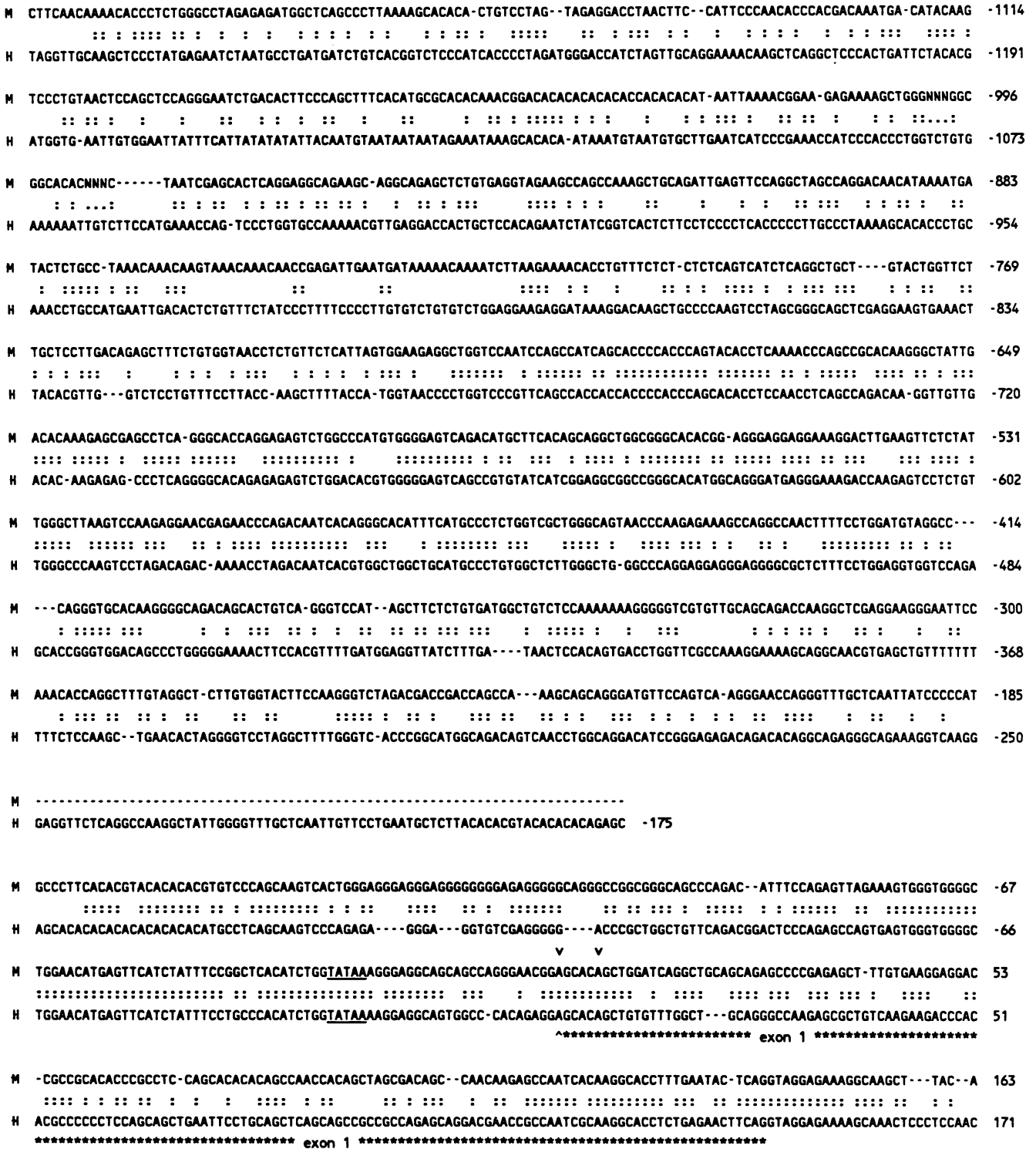


FIG. 3. Sequence similarity between 5' noncoding regions of the murine and human PAI-1 genes. The human sequence and mRNA cap designation are from Bosma et al. (3). The asterisks designate exon 1, and the carats indicate the mRNA caps sites; the TATAA consensus is underlined.

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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