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TRANSCRIPTIONAL REGULATION OF THE MOUSE PERFORIN GENE BY A PROXIMAL PROMOTER AND AN INTRONIC SILENCER

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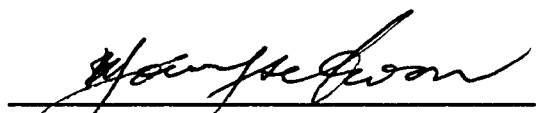

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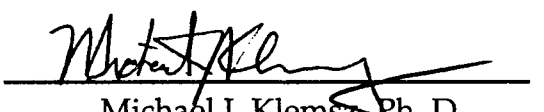

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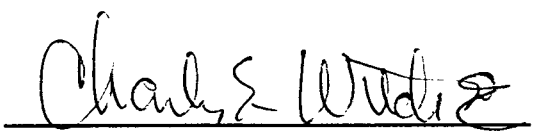

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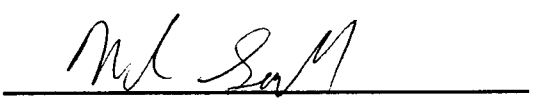

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Abstract

This study was designed to determine the potential regulatory elements involved in the transcriptional regulation of the mouse perforin promoter. DNase I hypersensitive site mapping (DHSM) revealed that the perforin promoter contained at least 6 DNase I hypersensitive sites (DHS) within 7.0 kb of 5' upstream sequence and 2 DHS in intron 2. The 5' upstream DHS and an intronic DHS were cytotoxic T-lymphocyte (CTL)-specific, while one of the intronic DHS was ubiquitously found.

A series of CAT plasmids were constructed and introduced into CTLL-R8, D10 (T helper), A20 (B cell lymphoma), and HeLa cells. CAT activity was detected only in CTLL-R8 cells, while no or minimal CAT activity was detected in D10, A20 or HeLa cells. Among these deletion constructs, a construct termed PFP5a, containing 795 bp 5' upstream sequence, exhibited the highest CAT activity in the CTLL-R8 cells. PFP9a20 containing 73 bp 5' upstream sequence also produced relatively higher CAT expression only in CTLL-R8. The proximal region in PFP9a20 contains two potential Sp1-binding sites (GC and GT box) and one canonical Ets-binding sites (EBS). Electrophoretic mobility shift assay (EMSA) showed that each element bound specific protein factors. When single-point mutation was introduced to the GC, GT box, and EBS in the proximal promoter as well as PFP5a, 2- to 3-fold less CAT activity was observed in CTLL-R8 cells. When PFP5a was subject to *in vitro* transcription in the presence of GC, GT box and EBS cold oligonucleotides, the level of transcription was decreased markedly. The results suggest that a combination of the three *cis*-acting elements may constitute a minimal region responsible for CTL-specific expression of the perforin gene.

To investigate a role(s) of the constitutive DHS (DHS VIII) in regulating the perforin gene, reporter-gene assays, EMSA, and DNase I footprinting assays were performed. These data suggested that DHS VIII exerted a common silencing effect on a variety of promoters and that the CTAT repeats within this region were responsible for the silencing activity and produced a specific DNA-protein complex. Since DHS VIII is composed of 73% AT-rich sequence and contains several HMG-I(Y) recognition sequences, the region appears to be associated with nuclear matrix-associated region (MAR). Since MAR are thought to be involved in transcriptional regulation, we propose that DHS VIII plays an important role in the perforin gene regulation.

Taken together, these two studies suggest that the mouse perforin gene may be regulated by positive regulatory elements residing in the proximal promoter and negative regulatory elements residing in intron 2.

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