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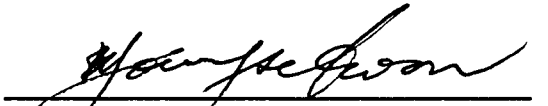
TRANSCRIPTIONAL REGULATION OF THE MOUSE PERFORIN GENE BY
A PROXIMAL PROMOTER AND AN INTRONIC SILENCER

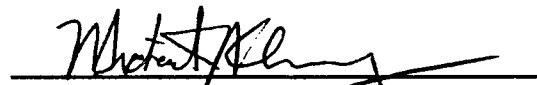
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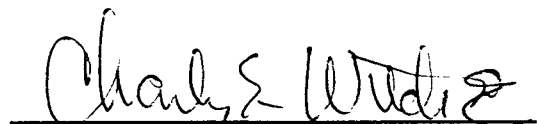
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Abstract

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.

TABLE OF CONTENTS

<u>Section</u>	<u>Page</u>
TITLE PAGE.....	i
ACCEPTANCE PAGE.....	ii
DEDICATION.....	iii
ACKNOWLEDGMENT.....	iv
ABSTRACT.....	v
TABLE OF CONTENTS.....	vii
INTRODUCTION	
T-cell development.....	2
T-cell activation.....	4
T-cell mediated immunity.....	5
The differentiation of CD4 T-cells into effector helper T-cells (Th).....	5
The differentiation of naive CD8 T-cells into cytotoxic T-lymphocytes (CTL).....	7
Perforin (pore forming protein or cytolysis).....	8
Biochemical properties of perforin.....	8
Perforin as an effector for granule exocytosis pathway.....	9
CTL utilizes Fas or TNF as perforin-independent mechanism.....	11
Perforin gene.....	13
Expression and regulation.....	13
Molecular analysis of perforin gene.....	14
Developmental study on T-cell differentiation at molecular level.....	15
Critical roles of Ikaros, GATA, TCF1, and Ets-1 in T lymphocyte Development.....	16

Summary.....	18
SPECIFIC AIMS.....	20
MATERIALS AND METHODS	
Cell culture.....	23
Preparation of splenic T-cells.....	23
DNase I hypersensitive site mapping.....	24
Plasmid preparation.....	26
Construction of reporter genes.....	26
Isolation of the mouse perforin intron 2 and construction of reporter genes.....	28
Transfection and reporter gene assay.....	30
Site-specific mutagenesis.....	31
Preparation of nuclear and whole-cell extract.....	31
Electrophoretic mobility shift assay (EMSA).....	32
<i>In vitro</i> transcription.....	34
DNase I foot printing assay.....	35
Yeast-one hybrid screen.....	36
construction of bait.....	36
Determination of His leakage.....	36
Construction of prey and selection.....	37
RESULTS	
The mouse perforin locus consists of at least 8 DNase I hypersensitive sites (DHS).....	39
Perforin gene expression is regulated by multiple enhancing and inhibitory elements.....	40

Expression of perforin reporter genes in perforin-positive and perforin-negative cells.....	41
Functional analysis of proximal 73-base promoter.....	42
The proximal GC-box, EBS, and GT-box are necessary for CTL-specific gene expression of the perforin gene. Evidence for the existence of CTL-specific Ets-related factor.....	44
Molecular cloning of transcription factors recognizing GC or EBS using yeast-one hybrid.....	47
Identification of a silencer element from the constitutive DHS (DHS VIII) in the mouse perforin intron 2.....	49
Localization of a silencing region DHS VIII.....	49
DHS VIII consists of high AT-rich sequence.....	50
DHS VIII functions as a general silencer.....	50
An intronic silencer map at the 3' 120 bp region of DHS VIII.....	51
CTAT repeats are responsible for the silencing activity.....	51

DISCUSSION

The mouse perforin gene locus contains CTL-specific DHS and non-CTL specific DHS.....	55
Role of 5' CTL-specific DHS.....	55
Role of the perforin intronic DHS.....	56
Proximal promoter elements, GC-box, EBS, and GT-box are important transcriptional regulators for mouse perforin gene expression.....	58
Role of proximal promoter.....	59
Role of Sp1 and Ets factor.....	61

The mouse perforin intron 2 plays an important role in regulating perforin gene expression.....	64
Is DHS VIII a nuclear matrix associating region (MAR)?.....	64
Role of the CTAT binding factor.....	66
Future experiments.....	67
Proposed models of regulation of the mouse perforin gene expression.....	68
Proximal promoter as a positive regulator.....	68
Perforin intron 2 may act as a genetic regulator in the chromatin and as a negative regulator.....	69

REFERENCES

FIGURES

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