RUNX Transcription Factor-Mediated Association of Cd4 and Cd8 Enables Coordinate Gene Regulation

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SUMMARY

T cell fate is associated with mutually exclusive expression of CD4 or CD8 in helper and cytotoxic T cells, respectively. How expression of one locus is temporally coordinated with repression of the other has been a long-standing enigma, though we know RUNX transcription factors activate the Cd8 locus, silence the Cd4 locus, and repress the Zbtb7b locus (encoding the transcription factor ThPOK), which is required for CD4 expression. Here we found that nuclear organization was altered by interplay among members of this transcription factor circuitry: RUNX binding mediated association of Cd4 and Cd8 whereas ThPOK binding kept the loci apart. Moreover, targeted deletions within Cd4 modulated CD8 expression and pericentromeric repositioning of Cd8. Communication between Cd4 and Cd8 thus appears to enable long-range epigenetic regulation to ensure that expression of one excludes the other in mature CD4 or CD8 single-positive (SP) cells.

INTRODUCTION

Cell fate is determined by complex patterns of gene expression that are often mediated by a surprisingly limited number of transcription factors. Within a particular lineage, key factors can both upregulate and repress the expression of different target genes, which can number in the hundreds and be scattered throughout the genome. How are these activities coordinated? Given that tissue-speciÞc expression proÞles can be accompanied by tissue-speciÞc patterns of locus conformation (Roldán et al., 2005; Sayegh et al., 2005; Skok et al., 2007) or nuclear location (near the nuclear periphery, pericentromeric heterochromatin [Brown et al., 1999], or within a chromosome territory [Chambeyron and Bickmore, 2004]), it is reasonable to ask whether

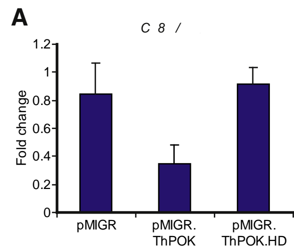
interaction between Cd4 and Cd8 occurs predominantly between loci located on the same chromosome.

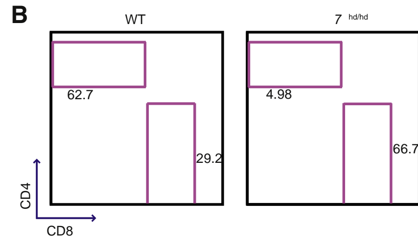
The $E8_I$ and $E8_{II}$ Enhancers Promote *Cd8* Transcription and *Cd4-Cd8* Association

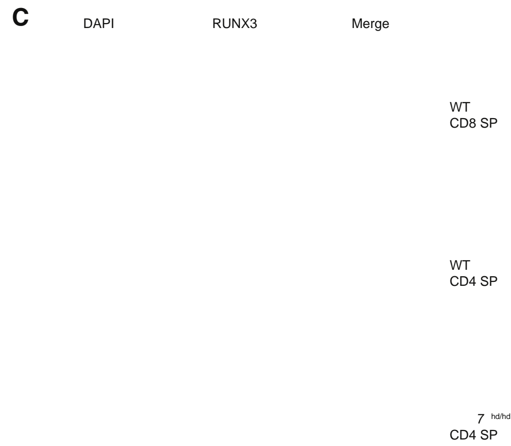
Having established that Cd4-Cd8 association occurs in Cd8-expressing cells, we turned our attention to regulatory elements in the Cd8 locus to determine how Cd8 transcription affects the relationship between the two loci. $CD8^+$ T cells express a heterodimer of CD8α and CD8β chains that are governed by at least Þve enhancer elements ($E8_I$ to $E8_V$; Figure S2A) that drive expression of CD8α and CD8β in a developmentally regulated manner (

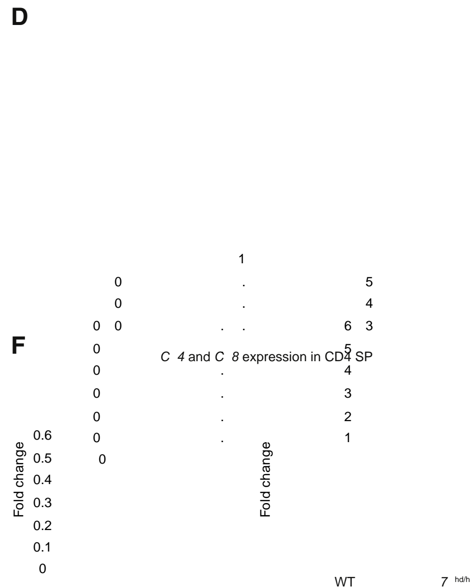
CD8 expression: $CD8^+$ $E8_IE8_{II}$ double-mutant DP cells showed signiÞcantly less Cd4-Cd8 association than did wild-type ($p = 9.13e-05$) and $CD8^{lo}$ $E8_IE8_{II}$ double-mutant DP cells showed even lower levels of Cd4-Cd8 association ($p = 3.33e-08$ compared to wild-type) (Figure 2D). Consistent with these results, RT-PCR analysis demonstrated that Cd8 transcription was lower than wild-type in $CD8^+$ $E8_IE8_{II}$ and almost abolished in $CD8^{lo}$ $E8_IE8_{II}$ double-mutant DP cells (Figure 2E).

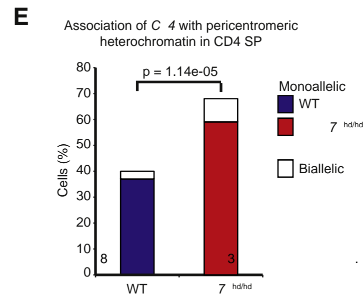
Repositioning of Cd8 to PCH was probably affected both by deletion of these enhancer elements and by the reduction in transcription. We observed increased positioning of Cd8 to PCH in the $CD8^+$ $E8_IE8_{II}$ double-mutant DP cells; about 52% of the double-mutant cells had at least one allele associated with











mice. Cd4 expression is regulated by a silencer element and at least one stage-speciÞc enhancer element (Chong et al., 2010; Kioussis and Ellmeier, 2002). The proximal enhancer Cd4 PE, located 13 Kb upstream of the Cd4 start site, is absolutely required for transcription, and therefore expression, of Cd4 in DP thymocytes (Chong et al., 2010). The position of this enhancer is diagrammed in Figure S5A. After positive selection in Cd4 proximal enhancer (PE)-deÞcient mice, CD4-expressing single-positive thymocytes and CD4^{+} peripheral T cells were detected, albeit at reduced numbers, and levels of CD4 expression were comparable to wild-type mice, suggesting that one or more putative enhancer elements rescue Cd4 expression (Figure 5A and data not shown). DNA FISH and confocal microscopy analysis of sorted thymocyte populations from Cd4 PE-deÞcient mice (Figure S2B) revealed that the Cd4 PE, and therefore Cd4 transcription, is not required for either the Cd4-Cd8 association at the DP stage or for the repositioning away from PCH, because the degree of Cd4-Cd8 association and

pericentromeric localization were comparable to wild-type (Figures 5BÐ5D; Table S4).

In contrast to wild-type cells, however, Cd4-Cd8 association in Cd4

population for real-time RT-PCR analysis. Cd4 transcription was virtually abolished in DP cells from Cd4 PE-deÞcient mice (data not shown) and substantially reduced in $CD4^{+}CD8$

in the DN thymocyte population and remained low at all subsequent stages of development ($p = 5.48e-13$ in DP cells, $p = 3.51e-03$ in $CD4^{+}CD8$

changes in gene activation and repression. As with most epigenetic correlations, this is a chicken-and-egg situation and we cannot pinpoint the initiating event.

These studies allow us to put forth the following model. The Cd4 and Cd8 loci come into close proximity in DP thymocytes. After positive selection, all thymocytes pass through a CD4$^+$ CD8^{lo} transitional stage in which Cd8 transcription decreases and it moves to pericentromeric regions, disrupting the Cd4-Cd8 association. In CD4-fated cells, ThPOK binds to the Cd4 silencer, preventing it from interacting again with the Cd8 locus. In CD8-fated cells, RUNX3 mediates the reassociation of Cd4 and Cd8 by binding to the Cd4 silencer and the Cd8 locus, predominantly within $E8_I$. Thus, RUNX-mediated Cd4-Cd8 association silences the Cd4 locus, repositioning it to repressive pericentromeric heterochromatin.

Although it has been known for some time that chromosomal interactions can exert an effect on gene expression in trans in Drosophila (transvection) (Lewis, 1985) and possibly plants (paramutation) (Stam, 2009), there are still only a few instances in which association of alleles is known to exert epigenetic control in mammals. Two examples involve the pairing of homologous alleles: X inactivation (Bacher et al., 2006; Xu et al., 2006) and allelic exclusion (Hewitt et al., 2009). Heterologous association between different loci has been noted in developing B cells as well: one immunoglobulin light chain (Igk) allele transiently associates with one immunoglobulin heavy chain (Igh) allele at pericentromeric regions, inducing a change in nuclear location and a conformational change within the Igh locus to prevent ongoing recombination (Hewitt et al., 2008). Similarly, association of different loci has been shown to occur in T cell subsets: the Ifng locus interacts with the Il4 locus just prior to commitment to either the Th1 or Th2 cell lineage, which express either IFN-γ or IL-4, respectively. The association of Ifng and Il4 could facilitate the coordinate regulation of these loci in the differentiated CD4$^+$ T cell subsets (Spilianakis et al., 2005) but no trans acting factors that could mediate the association have been identiÞed. Clearly this is an underexplored area of epigenetic regulation.

Our Þndings add to a growing body of evidence that nuclear architecture plays a dynamic role in regulating gene expression (Fraser and Bickmore, 2007). That association of Cd4-Cd8 is conserved in both mouse and humans, despite being located on different chromosomes in the latter, underscores the importance of this mechanism for regulating CD4 and CD8 coreceptor expression. Undoubtedly, a fuller understanding of the mechanism of Cd4-Cd8 association will yield insight into how these coreceptors are regulated during T cell development and how long-range chromosomal interactions control gene expression.

EXPERIMENTAL PROCEDURES

Mice

C57Bl/6 mice were purchased from Jackson Laboratories or Taconic. Cd4 PE-deÞcient (Chong et al., 2010), Cd4 sil-deÞcient (Zou et al., 2001), Cbfb$^{F/F}$ (Naoe et al., 2007), $E8_I$-deÞcient (Ellmeier et al., 1998), $E8_I E8_{II}$ double-mutant (Ellmeier et al., 2002), Lck-cre (Lee et al., 2001), and ThPOK transgenic (Sun et al., 2005) mice have previously been described. Mice were housed

Leica software. At least three independent experiments were performed (n = 166 to 356 alleles for Cd4-Cd8 association, see Supplemental Tables for one representative experiment of PCH analysis). Distances between the center of the Cd4 and Cd8 signals was measured with Image J software.

The empirical interallelic distance distributions were compared to test whether they had been drawn from the same underlying continuous distribution. The statistical signiÞcance of pair-wise distributionsÕ dissimilarity was assessed with the nonparametric two-sample Kolmogorov-Smirnov (KS) test (Massey, 1951). The reported p values were calculated with MATLAB 7.9 (The MathWorks Inc., Natick, MA).

Association of Cd4 and Cd8 with pericentromeric domains was scored if the loci signals were juxtaposed or overlapping with γ-satellite signals. Statistical signiÞcances for PCH localization were calculated with χ^2 test (Campbell, 1989). YatesÕ correction was applied when any category had less than 10 observations. Each data set was paired with the most relevant stage, genotype, or cell type.

RT-PCR

RNA was extracted with TRIZOL (Invitrogen). Reverse transcription was performed with Superscript III (Invitrogen), cDNA analyzed in triplicate with Quantitect Multiplex PCR Mix (QIAGEN) for Taqman probes or iQ SYBR Green Supermix (BioRad) in the iCycler (BioRad), and normalized to

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