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Induction of Germline Transcription in the Human TCRγ Locus by STAT5

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TCR and Ig genes are assembled by V(D)J recombination during lymphocyte development. The enhancer and the germline promoter control the accessibility of each locus for the common recombinaise activity. In the mouse TCRγ locus, STAT5 proteins activated by the IL-7R interact with consensus motifs in 5′ regions of Jγ segments and induce germline transcription. To evaluate the role of STAT5 in controlling the accessibility of the TCRγ locus, we characterized the germline transcription of human TCRγ genes and compared it with mouse. We first demonstrated that Jγ-Cγ germline transcripts are induced in a cytokine-dependent human erythroleukemia cell line. STAT consensus motifs are present in 5′ regions of Jγ1.1 and Jγ2.1 gene segments, and activated STAT5 binds to these motifs. By using a reporter assay, we showed that the Jγ1.1 germline promoter is transactivated by STAT5 and that mutations in any of the two STAT motifs abrogate this activity. Thus, this study demonstrates that STAT5 induces germline transcription in the TCRγ locus of both mouse and human and suggests the possibility that this mechanism may play an essential role in controlling the TCRγ locus accessibility. In addition, STAT motifs are conserved among 5′ Jγ germline promoters, 3′ enhancers, and a locus control region-like element, Hsa, in both mouse and human TCRγ loci, indicating the possibility that IL-7R/STAT5 signaling probably controls the locus-wide accessibility through these elements. The Journal of Immunology, 2001, 167: 320–326.

T cell receptor and Ig variable region genes are assembled during lymphocyte development from V, D, and J segments by V(D)J recombination. V(D)J recombination is conducted by conserved recombination signal sequences and recombinaise-activating gene-1 and -2 proteins. In developing T and B cells, specific molecular mechanisms exist that target the common recombinaise activity to appropriate TCR or Ig loci in a lineage-specific, developmentally ordered, and allelically excluded manner. This recombinaise targeting should occur at the level of the substrate locus, leading to a concept known as recombinational accessibility (1–3). Two kinds of cis chromatin elements are involved in controlling the accessibility. First, the enhancer elements govern the general accessibility of each locus in V(D)J recombination. Targeted deletion of the respective enhancers abolishes rearrangements in the TCRγ locus and greatly reduces rearrangements in the IgH, Igκ, and TCRα loci (2, 3). Histone acetylation is tightly correlated with V(D)J recombination in the TCRαδ and TCRβ/loci and is proposed as a mechanism for coupling enhancer activity to accessibility (4, 5). Second, the promoters for germline transcription control the local accessibility. T early α germline transcription takes place in the 5′ region of the Jα cluster in immature thymocytes. Targeted deletion of the T early α promoter results in severe impairment of the rearrangement of the 5′-most Jα segments (6). In another case, targeted deletion of the δβ1 germline promoter abolishes δβ1 germline transcription and reduces the rearrangement of the Dβ1 gene segment (7). These results suggest a critical role for germline transcription in V(D)J recombination of the Ag receptor genes.

IL-7 is an essential cytokine for early lymphocyte development when V(D)J recombination takes place. IL-7 exerts its effect through interaction with the IL-7R, consisting of a unique α-chain (IL-7Rα) and the common cytokine receptor γ-chain. Injection of neutralizing Abs to IL-7 or IL-7Rα, or genetic ablation of IL-7, IL-7Rα, or common cytokine receptor γ-chain, leads to a block of lymphocyte development. Although IL-7Rα-deficient mice have small numbers of B cells and αβ T cells in the periphery, they totally lack γδ T cells (8–10). The IL-7R transmits two signals in lymphocyte progenitors (11). One is for survival and proliferation. For instance, IL-7R signaling induces the expression of Bcl-2 in T cell precursors (12), and introduction of a bcl-2 transgene restores αβ T cell development in IL-7Rα-deficient mice (13, 14). The IL-7R also promotes the proliferation of lymphocyte precursors through the activation of phosphatidylinositol 3 kinase (15, 16). The other signal from the IL-7R is to promote V(D)J recombination in the IgH and TCRγ loci. For example, IL-7R signaling induces germline transcription and DNA rearrangement in D-distal V segments in pro-B cells (17). The V-J recombination and germline transcription of TCRγ genes also are severely impaired in IL-7Rα-deficient mice (18–21).

IL-7 binding to IL-7R triggers the phosphorylation and activation of receptor-associated Jak1 and Jak3 tyrosine kinases (22). After their activation, the Jak kinases phosphorylate the tyrosine residue of IL-7Rα.STAT, as well as phosphatidylinositol 3 kinase 2 domain-mediated interactions and translocate into the nucleus. Dimerized STAT proteins then bind to a consensus binding motif (TTCNNNGAA) and activate

3 Address correspondence and reprint requests to Dr. Koichi Ikuta, Department of Medical Chemistry, Graduate School of Medicine, Kyoto University, Yoshida, Sakyo-Ku, Kyoto 606-8501, Japan. E-mail address: ikuta@mfour.med.kyoto-u.ac.jp

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the transcription of various target genes. STAT5 and STAT1 interact with Nmi, an N-Myc interactor. Nmi enhances the association of CBP/p300 transcriptional coactivator proteins with STAT5 and STAT1 to augment IL-2- and IFN-γ-dependent transcription (23).

The IL-7R is required for the TCR locus accessibility (24). STAT5 proteins activated by the IL-7R interact with consensus motifs in 5' regions of Jy segments and induce germline transcription (21). A constitutively active form of STAT5 induces germline transcription, restores V-J recombination of TCRγ genes, and rescues γδ T cell development from IL-7R−/− T cell precursors. These results suggest that STAT5 controls the accessibility of the TCRγ locus by the induction of germline transcription. The transcriptional coactivators have intrinsic histone acetyltransferase activity and have been suggested to be involved in the accessibility control of the transcriptional machinery (25). Thus, it is conceivable that these coactivators render the chromatin accessible not only to the transcriptional machinery but also to the recombinational machinery. STAT5 may recruit the transcriptional coactivators to the Jγ regions and thereby regulate the recombinational accessibility in the TCR loci, especially at the Jγ regions.

To evaluate the STAT5-induced germline transcription of the TCRγ locus, we characterized the germline transcription of human TCRγ genes and compared it with mouse. Cytokine stimulation leads a human hemopoietic cell line to induce phosphorylation of the TCRγ locus. Activated STAT5 proteins interact with consensus motifs in 5' regions of Jy segments and induce germline transcription. These results are basically the same with those in the mouse TCRγ locus. Thus, this study demonstrates that STAT5 induces germline transcription in the TCRγ locus of both human and mouse and suggests the possibility that this mechanism may play an essential role in controlling the accessibility of the TCRγ locus. In addition, STAT motifs are conserved among 5' Jy germline promoters, 3' enhancer of the TCRγ locus (Eγ), and a locus control region-like element, HsA, in both mouse and human TCRγ loci, indicating the possibility that IL-7R/STAT5 signaling probably controls the locus-wide accessibility through these elements. We will also discuss its evolvement implication in αβ and γδ T cell development.

Materials and Methods

Cells

A mouse IL-3-dependent pro-B cell line, Ba/F3, was cultured as described previously (21). A GM-CSF-dependent human erythroleukaemia cell line, TF-1 (26), was maintained in RPMI 1640 medium containing 10% FBS and 5 ng/ml recombinant human GM-CSF (Genzyme, Cambridge, MA).

Northern blot analysis

Total RNA was electrophoresed through 1% agarose gel and transferred to a nylon membrane (Hybond-N; Amersham Pharmacia, Arlington Heights, IL). The membrane was hybridized sequentially with 32P-labeled Cy3 and GAPDH probes. The Cy3 probe was a 530-bp human Cγ1 (hCγ1) fragment isolated by PCR with primers as follows: 5'-AGACAATCTCTGTAATCTTTCCC-3' (probe A); 5'-ATCAATTACGAGAAAGAGATTACAGAATTGCT-3' (probe B); 5'-ATCAATTACGAGAAAGAGATTACAGAATTGCT-3' (probe B); 5'-ATCAATTACGAGAAAGAGATTACAGAATTGCT-3' (probe B); 5'-ATCAATTACGAGAAAGAGATTACAGAATTGCT-3' (probe B). The mutated probe contained a mutated motif (TTCNNTC) instead of the consensus motif (TTCCNGGA) of the atypical motif (TTCCNGTA).

Isolation of human 5' Jγ regions

The human 5' Jγ1 and 5' Jγ2.1 fragments were cloned by PCR. These fragments are the 1.0-kb 5' Jγ1 and 1.3-kb 5' Jγ2.1 regions that cover the sequences just before the first ATG of the germline transcripts. The 5' Jγ2.1 region contains an Alu repetitive sequence. Mutations were introduced in STAT5 consensus motifs by PCR-based mutagenesis with an LA-PCR in vitro mutagenesis kit (Takara Shuzo, Kyoto, Japan). The 5' end of the germline Jy-Cγ transcript was determined by cloning and sequencing its cDNA from TF-1 cells with a 5' Full RACE kit (Takara). Sequences of the primers for isolation of human 5' Jγ regions are as follows: 5'-GAGACGTCGAAGAGATCAGAATTGCT-3'; 5'-GAGACGTCGAAGAGATCAGAATTGCT-3'; 5'-GAGACGTCGAAGAGATCAGAATTGCT-3'; 5'-GAGACGTCGAAGAGATCAGAATTGCT-3'. The mutated probe contained a mutated motif (TTC NNTC) instead of the consensus motif (TTCCNGGA) of the atypical motif (TTCCNGTA).

Luciferase reporter gene transactivation assay

Transfection was done by electroporation as described previously (21). A reporter construct with mouse 5' Jγ1 germline promoter was described before (21). Ba/F3 cells were transiently transfected by electroporation with 10 μg of luciferase reporter plasmids driven by the 1.0-kb 5' Jγ1 or 1.3-kb 5' Jγ2.1 fragment (pGL2; Promega, Madison, WI) and 1 μg of a β-galactosidase plasmid driven by the Rous sarcoma virus long-terminal repeat promoter (pRSV-β-gal) as well as 10 μg of a STAT5A vector (pMX-STAT5A). The total amount of DNA was kept constant with pGL2-promoter constructs and in β-galactosidase vector (Stratagene, La Jolla, CA). Sequences of the primers are as follows: 5'-GAGACGTCGAAGAGATCAGAATTGCT-3'; 5'-GAGACGTCGAAGAGATCAGAATTGCT-3'; 5'-GAGACGTCGAAGAGATCAGAATTGCT-3'. The mutated probe contained a mutated motif (TTCNNTC) instead of the consensus motif (TTCCNGGA) of the atypical motif (TTCCNGTA).

Comparison of DNA sequences between the human and mouse TCRγ locus

Nucleotide sequences of the TCRγ locus were compared between human (GenBank accession no. AF159056) and mouse (GenBank accession no. AF037352) by GENETYX-MAC version 7.3 software (Software Development, Tokyo, Japan).
Results

Cytokine stimulation augments germline transcription of the human TCRγ locus

We and others have reported previously that TCRγ genes are frequently rearranged in the mouse and human thymus (18, 27–30). These results suggested that Vγ-Jγ recombination takes place in the majority of αβ T cells in the thymus. Germline transcription from unrearranged alleles usually precedes V(D)J recombination and is considered as an index of locus accessibility (1). To explore the mechanism of accessibility control of the human TCRγ locus (Fig. 1A), we examined germline transcription in a human erythroleukemia cell line, TF-1, by Northern blot analysis (Fig. 1B). TF-1 cells proliferate depending on erythropoietin, GM-CSF, or IL-3 and have the TCRγ locus in germline configuration (data not shown). Because stimulation by these cytokines activates STAT5 (22), we had thought that TF-1 cells may induce germline transcripts of TCRγ genes as we reported previously with a mouse IL-3-dependent cell line, Ba/F3. A high level of germline transcripts was detected in TF-1 cells cultured with GM-CSF. When deprived of GM-CSF, TF-1 cells slightly down-regulated the level of the transcripts. The levels of the TCRγ transcripts was found to be almost the same in TF-1 cells cultured with or without cytokine stimulation (Fig. 2A). GM-CSF signaling augments germline transcription of TCRγ genes, even though the induction is not tightly controlled by the cytokine.

Next we examined the STAT5 activation in TF-1 cells cultured with or without GM-CSF (Fig. 1C). GM-CSF receptor signaling usually activates and phosphorylates JAK2 and STAT5 proteins. STAT5 protein was immunoprecipitated with anti-STAT5 Ab and immunoblotted with anti-phosphotyrosine or anti-STAT5 Ab. STAT5 was tyrosine-phosphorylated after GM-CSF stimulation. Before stimulation, STAT5 was not phosphorylated at all, even though STAT5 molecules were detected before and after GM-CSF stimulation. This result confirmed that STAT5 is activated by the GM-CSF stimulation in TF-1 cells. Taken together, these results indicated that germline transcription in the human TCRγ locus is induced in both cytokine-dependent and -independent manners in TF-1 cells.

STAT consensus motifs are conserved in the 5′ regions of Jγ1.1 and Jγ2.1 gene segments

To elucidate the mechanism of TCRγ germline transcription, we characterized the promoter regions for the transcription. We checked the sequence of 5′ regions of five Jγ gene segments (Fig. 2). A STAT consensus motif (TTCNNNGAA) was found at ~300 bp upstream of the Jγ1.1 gene segment. By sequence homology, we noticed a similar STAT consensus motif at ~600 bp upstream of the Jγ2.1 gene segment. An Alu repetitive sequence was inserted between the STAT motif and the Jγ2.1 gene segment. We also found second atypical STAT motif (TTCNNNGTA) at 5 bp downstream of the first motifs in 5′ Jγ1.1 and 5′ Jγ2.1 regions.

We next determined transcription initiation sites of TCRγ germline transcripts by rapid amplification of cDNA ends method. TF-1 cells transcribed two kinds of germline transcripts, Jγ1.1-Cγ1 and Jγ1.1-Cγ2.1 gene segments.

**FIGURE 1.** Germline transcription in the human TCRγ locus. A, The schematic illustration of the human TCRγ locus. Exons and pseudogenes are depicted as ■ and □, respectively. P represents a pseudogene. B, Induction of germline transcripts of TCRγ genes in a human erythroleukemia cell line. Total RNA was isolated from TF-1 cells cultured with (+) or without (−) GM-CSF, and a Northern blot was hybridized sequentially with Cγ1 and GAPDH probes. C, Phosphorylation of STAT5 by GM-CSF stimulation. Total cellular extract was isolated from TF-1 cells with (+) or without (−) cytokine stimulation and immunoprecipitated with anti-STAT5 Ab. Western blots were visualized sequentially with anti-phosphorylated STAT5 and anti-STAT5 Abs.

**FIGURE 2.** STAT consensus motifs conserved in human 5′ Jγ regions. A, STAT consensus motifs in two 5′ Jγ regions. STAT consensus motifs in the 5′ end of Jγ1.1, Jγ2.1, and the region used for reporter constructs are boxed. P represents a pseudogene. B and C, DNA sequence of 5′ Jγ1.1 (B) and 5′ Jγ2.1 (C) regions. STAT consensus motifs, the nonamer and heptamer recombination signals, and an Alu repetitive sequence are shown by arrows.
Jy2.1-Cy2. The Jy1.1-Cy1 transcripts started from two sites of ~100 and 110 bp downstream of the first STAT motif in 5’ Jy1.1 region (Fig. 2B). This distance between the motifs and transcription initiation sites is typical of STAT5-dependent promoters. The Jy2.1-Cy2 transcripts started from two sites of ~70 bp (from within the Alu sequence) and 400 bp downstream of the first STAT motif in 5’ Jy2.1 region (Fig. 2C). Either of them may have started under the influence of promoter activity within the Alu sequence.

To distinguish the Jy1.1-Cy1 and Jy2.1-Cy2 transcripts, we designed PCR primers common to both, and amplified and subcloned cDNA of TF-1 cells cultured with and without GM-CSF. DNA sequences of randomly picked up clones were determined and compared (Table I). The Jy1.1-Cy1 transcripts were the majority of the germline transcripts in TF-1 cells before and after cytokine stimulation. This result suggested that the insertion of Alu sequence and other changes probably diminished the promoter activity of the 5’ Jy2.1 region.

STAT5 binds to the consensus motifs in 5’ Jy regions

The coincidence of the STAT consensus motifs in two different 5’ Jy regions led us to the idea that STAT proteins, activated by IL-7R signaling, bind to these motifs and induce germline transcription as in the mouse (21). To test this, we first analyzed the binding of STAT proteins to the motifs by EMSA (Fig. 3). Because DNA sequences around the STAT consensus motifs are highly conserved between 5’ Jy1.1 and 5’ Jy2.1 regions, we designed oligonucleotide probes to 5’ Jy1.1 region (Fig. 3A). IL-3 stimulation induced binding activity to the oligonucleotide probe A corresponding to the typical motif in the 5’ Jy2.1 region, in Ba/F3 cells, similarly to a control mouse probe (Fig. 3B). By incubation with anti-STAT5 Ab, this activity showed a supershift. Mutation in the motif deprived the binding capacity of the probe. To test whether STAT5 also can bind to the second atypical motif, we next conducted EMSA with the oligonucleotide probe B that covers two STAT motifs (Fig. 3C). Strong binding activity was detected after IL-3 stimulation and showed a supershift by anti-STAT5 Ab. Single mutation in the first typical motif did not affect the interaction. Double mutations in the first and the second motifs completely abrogated the binding capacity of the probe. The interaction of STAT5 proteins with the second motif was unequivocally proved by the oligonucleotide probe C, which covers only the second motif (Fig. 3D).

STAT5 transactivates the germline promoters of the human TCRγ locus

Next we checked by luciferase reporter assay whether the binding of STAT5 to the motifs results in the induction of transcription (Fig. 4). We used Ba/F3 cells because they give a higher level of transfection efficiency than other cell lines. A 1.0-kb and a 1.3-kb fragment of 5’ Jy1.1 and 5’ Jy2.1 regions, respectively, were joined to a luciferase reporter gene (Fig. 4A). These plasmid DNAs were electroporated into Ba/F3 cells together with an expression plasmid coding for STAT5A. The cells were starved for 6 h and then restimulated with IL-3. Cytokine stimulation alone induced Stat5- and IL-3-dependent transactivation in Ba/F3 cells by luciferase reporter assay (Fig. 4B). With exogenous STAT5A this induction was augmented about threefold. The human 5’ Jy1.1 promoter showed similar pattern of STAT5-dependent induction, suggesting that STAT5 can transactivate this promoter. In contrast, the 5’ Jy2.1 promoter revealed relatively higher levels of reporter expression even without exogenous STAT5. In addition, induction by cytokine stimulation was less drastic. This result suggested that the 5’ Jy2.1 region has lost STAT5-dependent promoter activity. To test whether the Alu sequence is responsible for the impaired activity, we next deleted the Alu sequence from the 5’ Jy2.1 fragment and similarly tested its promoter activity (Fig. 4B, 5’Jy2.1ΔAlu). The deletion of the Alu sequence did not result in any significant change of the promoter activity. This result suggested that the promoter activity of the 5’ Jy2.1 region was diminished mainly by changes other than insertion of the Alu sequence.

We next checked whether the conserved motifs are important for this transactivation. Different sets of mutations were introduced in the motifs of the 5’ Jy1.1 promoter, and these were tested for Stat5- and IL-3-dependent transactivation in Ba/F3 cells by luciferase reporter assay (Fig. 4C). Single mutations in any of the two motifs caused dramatic decreases in transcription mediated by exogenous Stat5A. Double mutations resulted in similar decrease to single mutations. This result suggested that the interaction of
STAT5 with a set of two intact motifs probably plays an essential role to activate germline transcription from the 5' Jg1.1 promoter.

Comparison of cis-control elements in the TCRγ locus between human and mouse revealed that STAT consensus motifs are conserved in 5' Jg1.1 and 3' enhancers, and a locus control region-like element, HsA (Fig. 5). Human 5' Jg1.1 and mouse 5' Jg1 germline promoters have relatively high sequence homology (61% for 600 bp; Fig. 5A). The first STAT motif in human is conserved as the third motif in mouse. Other STAT motifs were not conserved. Homology search for other transcription

FIGURE 4. Transcriptional activation of 5' Jγ1 promoter by STAT5. A, Schematic illustration of luciferase reporter constructs. The 1.0-kb 5' Jγ1.1 and 1.3-kb 5' Jγ2.1 fragments with or without mutated motifs and Alu sequence deletion, respectively, were flanked by luciferase cDNA. B, Transcriptional activation of 5' Jγ1.1 and 5' Jγ2.1 fragments by STAT5. Ba/F3 cells were transfected with the mixture of the luciferase reporter plasmids, STAT5A expression vector, and β-galactosidase control vector, and stimulated with or without IL-3. Luciferase activity in the whole cell lysate was normalized with β-galactosidase activity. Data are the mean ± SE of triplicate data points from a representative experiment. C, Full transactivation of 5' Jγ1.1 fragment requires two intact consensus motifs. Promoter activity of the 5' Jγ1.1 fragments carrying single or double mutations in the motifs was determined as described in B.

STAT5 with a set of two intact motifs probably plays an essential role to activate germline transcription from the 5' Jγ1.1 promoter.

Conserved STAT motifs in the human and mouse TCRγ loci

Comparison of cis-control elements in the TCRγ locus between human and mouse revealed that STAT consensus motifs are conserved in 5' Jγ1 germline promoters, 3' enhancers, and a locus control region-like element, HsA (Fig. 5). Human 5' Jγ1.1 and mouse 5' Jγ1 germline promoters have relatively high sequence homology (61% for ~600 bp; Fig. 5A). The first STAT motif in human is conserved as the third motif in mouse. Other STAT motifs were not conserved. Homology search for other transcription

FIGURE 5. Comparison of cis-control elements in the human and mouse TCRγ loci. Human and mouse 5' Jγ1 germline promoters (A), 3' enhancers (B), and HsA elements (C) were compared. (−), aligned identical bases and gaps are indicated (−). Conserved motifs for transcription factor and the nonamer and heptamer recombination signals are shown by boxes. Germline transcription and Jγ genes are shown by arrows. The DNase footprints (NF-y1 to NF-y6) of mouse 3' enhancer are underlined in B. The consensus transcription factor binding sites in mouse HsA are underlined in C.
factor binding sites failed to find any site in common between human and mouse. The human and mouse Eγ also have high sequence homology (57% for ~750 bp; Fig. 5B). As previously reported (31), the region from NF-γ2 to NF-γ4 sites is highly conserved between human and mouse (74% for ~140 bp). In the NF-γ2 site, a STAT consensus motif is conserved. A PEBP2 binding site also is conserved in the NF-γ3 site.

A previous report suggested that a DNase I hypersensitive site between Vγ5 and Vγ2 genes serves as a locus control region in concert with Eγ (32). Comparison between human and mouse Vγ regions demonstrated that human Vγ1.8 and mouse Vγ5 and human Vγ4P and mouse Vγ2 have sequence homology (data not shown). Besides these, mouse HsA element have a homologous region in human between Vγ2 and Vγ3P genes (59% for ~510 bp; Figs. 5C and 6). Thus, the relative location of HsA also is conserved between human and mouse. A STAT motif and a p300 motif are conserved in human and mouse HsA. However, other GATA-3, GAGA, LEF/TCF, E box, and myb motifs in the mouse HsA are not conserved in human. A 2-kb DNA region including mouse HsA also has 50–60% sequence homology to a DNA region between human Vγ2 and Vγ3P (data not shown). Thus, these results demonstrated phylogenetic conservation of STAT consensus motifs in three types of cis-control elements in the TCRγ locus.

Discussion
In this study, we first demonstrated that Jγ-Cγ germline transcripts are induced in a GM-CSF-dependent human erythroleukemia cell line (Fig. 1). STAT consensus motifs are present in 5′ regions of Jγ1.1 and Jγ2.1 gene segments (Fig. 2), and activated STAT5 binds to these motifs (Fig. 3). By a reporter gene assay, we showed that the promoter of Jγ1.1 germline transcription is transactivated by STAT5 and that mutations in the STAT consensus motifs abolish this activity. The 5′ Jγ2.1 region have lost STAT5-dependent promoter activity, probably because of changes other than insertion of the Alu sequence (Fig. 4). These results basically are the same with those in the mouse TCRγ locus (21). Thus, this study demonstrates that STAT5 induces germline transcription in the TCRγ locus of both mouse and human, and suggests the possibility that this mechanism may play an essential role in controlling the accessibility of the TCRγ locus.

STAT motifs are conserved among 5′ Jγ germline promoters, Eγ elements, and a locus control region-like element, HsA, in both mouse and human TCRγ loci (Figs. 5 and 6). This evidence indicates that these cis-control elements altogether cooperate to regulate the accessibility of the TCRγ locus through their possible interaction with STAT5. First, it is probable that the Eγ elements control the general accessibility of the locus, as has been described for other TCR and Ig loci (2, 3). Second, the HsA element also is likely to contribute to the locus-wide accessibility (32). Finally, as characterized in the previous (21) and this study, STAT5 induces germline transcription and regulates the local accessibility near Jγ gene segments by histone acetylation (S.K.Y. and K.I., unpublished data).

Enhancers and promoters can direct the hyperacetylation of core histones because of histone acetyltransferase activity of transcriptional coactivators. Histone acetylation is tightly correlated with V(D)J recombination in the TCRαβ and TCRγδ loci and is proposed as a mechanism for coupling enhancer activity to accessibility (4, 5). In the TCRγ locus, histones are hyperacetylated at transcriptionally active Jγ segments in normal but not in IL-7Rα-deficient thymocytes precursors. Interestingly, the acetylation levels of Eγ region also are high in normal but low in IL-7Rα-deficient thymocytes (S.K.Y. and K.I., unpublished data). Interaction of STAT5 with Eγ may recruit transcriptional coactivators and induce histone acetylation. It will be interesting to elucidate the role for STAT5 in locus-wide accessibility control of the TCRγ locus through the Eγ and HsA elements.

The phylogenetic conservation of STAT consensus motifs in three types of cis-control elements indicates that the TCRγ locus is under the strong influence of IL-7R and STAT5 signaling. In contrast, the STAT motifs are not conserved in enhancers and germline promoters of the other TCR loci. After entry into the thymus, T cell precursors first proliferate by stimuli from c-kit and the IL-7R. At this stage, they receive a signal from the IL-7R to induce the rearrangement and transcription of the TCRγ locus. This will help them to commit and maintain themselves to the γδ T cell lineage. However, at later stages of αβ T cell development, this signal for γδ T cells seems to be shut off. We speculate that pre-TCR signaling may cancel this IL-7R signal to facilitate the differentiation into the αβ T cell lineage. During evolution of the immune system, γδ T cells may have emerged first with the simple mechanism that IL-7 produced from epithelial cells of the skin and intestine induces the V(D)J recombination and cell expansion. αβ T cells probably evolved later with a more sophisticated system where pre-TCR signaling invalidates the IL-7R signal enabling positive and negative selection to operate on the basis of interactions between αβ TCRs and self-MHC.

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