Differential Expression of CD45 Isoforms Is Controlled by the Combined Activity of Basal and Inducible Splicing-regulatory Elements in Each of the Variable Exons*

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The human CD45 gene encodes five isoforms of a transmembrane tyrosine phosphatase that differ in their extracellular domains as a result of alternative splicing of exons 4–6. Expression of the CD45 isoforms is tightly regulated in peripheral T cells such that resting cells predominantly express the larger CD45 isoforms, encoded by mRNAs containing two or three variable exons. In contrast, activated T cells express CD45 isoforms encoded by mRNAs lacking most or all of the variable exons. We have previously identified the sequences within CD45 variable exon 4 that control its level of inclusion into spliced mRNAs. Here we map the splicing-regulatory sequences within CD45 variable exons 5 and 6. We show that, like exon 4, exons 5 and 6 each contain an exonic splicing silencer (ESS) and an exonic splicing enhancer (ESE), which together determine the level of exon inclusion in naive cells. We further demonstrate that the primary activation-responsive silencing motif in exons 5 and 6 is homologous to that in exon 4 and, as in exon 4, binds specifically to the protein heterogeneous nuclear ribonucleoprotein L. Together these studies reveal common themes in the regulation of the CD45 variable exons and provide a mechanistic explanation for the observed physiological expression of CD45 isoforms.

In order to attain proteomic and functional complexity, higher eukaryotes have developed numerous mechanisms to amplify the informational content of their relatively limited genomes. One such mechanism, alternative splicing, allows for the production of multiple unique mRNAs from a single gene through the differential inclusion of exons. Since the variant mRNAs produced by alternative splicing can each potentially encode for a functionally distinct protein, alternative splicing is recognized to be a ubiquitous and critical mechanism for regulating cellular function (1, 2).

The importance of regulated alternative splicing in humans is exemplified by the CD45 gene, which encodes a transmembrane protein-tyrosine phosphatase (3). CD45 phosphatase activity is critical for intracellular signaling in T cells in response to antigen stimulation, as indicated by the severe immunodeficiency observed in CD45-deficient mice and humans (4–7). Five isoforms of the CD45 protein are expressed in humans as a result of alternative inclusion of exons 4–6 (see Fig. 1A). In human T cells, the expression of the CD45 isoforms is tightly regulated throughout development and upon activation (3). In particular, naive peripheral T cells express significant amounts of the larger isoforms of CD45, which have high phosphatase activity and maintain the T cell receptor in a state primed for antigen recognition (8). Subsequent to antigen stimulation, however, there is a marked shift in the processing of CD45 pre-mRNA, such that the variable exons are predominantly excluded from the final message, resulting in expression of the smaller CD45 isoforms (9, 10). The smaller isoforms of CD45 are more prone to homodimerization than the larger isoforms (8, 11). Importantly, in the dimeric state the intracellular phosphatase activity of CD45 is inhibited by steric constraints (8). Therefore, the shift to smaller CD45 isoforms upon activation of T cells reduces CD45 phosphatase activity and is thought to contribute to the attenuation of T cell signaling (8, 12). Such a model for the importance of CD45 alternative splicing in maintaining T cell homeostasis is supported by studies that have correlated defects in CD45 splicing regulation with susceptibility to various autoimmune diseases, including multiple sclerosis, systemic sclerosis, and autoimmune hepatitis (13–15).

To fully understand and predict the differential expression of CD45 isoforms in T cells, we need a complete characterization of the determinants of CD45-regulated splicing. As for most examples of alternative splicing, the splice site sequences at the intron-exon boundaries surrounding the CD45 variable exons deviate significantly from the consensus recognition sites for the splicing machinery or “spliceosome” (16).

Thus, auxiliary sequences (i.e. enhancers or silencers) within or flanking the variable exons are likely to play a critical role in determining the efficiency of spliceosome binding and the resulting exon inclusion. Despite a general appreciation of the role of enhancers and silencers in alternative splicing, our ability to predict the presence and activity of such regulatory elements remains limited (17). Therefore, experimental dissection of CD45 variable exons is required to identify the regulatory motifs that influence CD45 isoform expression. Previously, we have identified the regulatory sequences that control inclusion of CD45 variable exon 4, including a motif referred to as the activation-responsive sequence (ARS) consensus that is required for exon silencing upon T cell activation (18, 19). In this study, we have extended our understanding of the splicing-regulatory sequences controlling CD45 isoform expression by systematically mapping all of the exonic regulatory sequences that control inclusion of CD45 exons 5 and 6 in resting and activated T cells. Consistent with the physiologic distribution of CD45 isoforms, we find that exon 5 contains a strong enhancer (ESE)3 and a weak silencer (ESS), whereas exon 6 contains two strong ESSs and a weaker ESE. In addition, we show that, as with exon 4, exons 5 and 6 each contain a sequence sufficient to confer activation-induced exon

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9 The on-line version of this article (available at http://www.jbc.org) contains supplemental Table I.
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3 The abbreviations used are: ESE, exonic splicing enhancer; ESS, exonic splicing silencer; RT, reverse transcription; ARS, activation-responsive sequence; PMA, phorbol 12-myristate 13-acetate; FR, -fold repression; hnRNP, heterogeneous nuclear ribonucleoprotein; PTB, poly(B)tract-binding protein.
Regulation of CD45 Exons 5 and 6

repression, which binds hnRNP L and obligatorily contains the ARS consensus motif. Taken together, we conclude that CD45 isoform expression is determined by the combinatorial effect of the basal and inducible regulatory activities encoded within each of the three variable exons.

EXPERIMENTAL PROCEDURES

**Minigenes**—Constructs CD9, SC5, SC6, and SC14 were described previously (19). Constructs CD5 and CD6 contain a BglII-flanked region of ~500 nucleotides of genomic sequence encompassing CD45 exons 5 and 6 that was removed from SC5 and SC6 and substituted for the exon 9 sequences in CD9. Constructs SC-E5 and SC-E6 were generated by inserting PCR-derived 120-nucleotide fragments internal to exons 5 and 6 into the GloI construct described previously (19). Block scanning mutations within SC5 and SC6 were done using PCR to swap blocks of 20 nucleotides within exon 5 to TCAGTATGACTCTCAGTATG or 15 nucleotides within exon 6 with TCAGTATGACTCTCA. Constructs shown in Figs. 4 and 5 to test single or double copies of individual regions of exons 5 and 6, were made by ligation of double-stranded DNA oligonucleotides corresponding to the given sequence (shown in figures) into the MluI site of SC-glo. Constructs E5:R1–5, E5:R1–5(mARS), E6:R5–8, and E6:R5–8(mARS) were also made by insertion of the corresponding synthetic oligonucleotides into the MluI site of SC-glo. SC-glo is identical to the construct “glo” described previously (20).

**In Vivo Splicing Analysis**—Transfections of minigenes, stable clone selection, stimulations, RNA isolation, and analysis of minigene splicing by RT-PCR were done as described previously (19).

**UV Cross-linking**—[32P]-Labeled RNA was generated by *in vitro* transcription with T7 polymerase (Promega) in the presence of [32P]CTP. Templates for *in vitro* transcription were generated by cloning double-stranded DNA oligonucleotides encoding E6R5–8 with or without mARS mutations or E5R1–5 with or without mARS mutations downstream of a T7 promoter in the vector pSPT7 (20). Generation of nuclear extract from JSL1 cells and UV cross-linking assays were done as described previously (20).

**RNA Interference**—Small interfering RNAs, transfection, and analysis by RT-PCR and Western blot were done identically to that described recently by Rothrock et al. (20).

RESULTS

The relative expression of the five CD45 isoforms in human peripheral T cells results from the differential inclusion of each of the three variable exons within the CD45 gene. Variable exons 4 and 6 are only included in a fraction (roughly 40–60%) of mRNAs expressed in resting T cells, whereas exon 5 is expressed in almost all of these mRNAs (10). Upon antigen stimulation, inclusion of exons 4 and 6 is barely detectable, whereas approximately half of the mRNAs still include exon 5 (10). This pattern of CD45 expression (diagrammed in Fig. 1A) suggests differences in the relative strengths of either the splice sites or auxiliary regulatory sequences within or surrounding exons 4 and 6 versus exon 5. In addition, whereas the inclusion of exons 4 and 6 is clearly repressed upon T cell activation, it has been debated whether the splicing of exon 5 is inherently regulated or is a stochastic event influenced by the neighboring effects of the other two variable exons (3).

To gain a deeper understanding of the mechanisms controlling CD45 isoform expression, we have sought to identify and characterize the sequences that determine inclusion of CD45 variable exons 4–6. Previously, we have identified an ESS and ESE within CD45 exon 4 that together result in the partial inclusion phenotype of this exon in resting T cells (18). The activity of the exon 4 ESS, but not ESE, is further enhanced upon T cell activation and is necessary and sufficient for the decreased inclusion of exon 4 in activated cells (19). Comparison of the sequence of the activation-inducible ESS within exon 4 with sequences in exons 5 and 6 indicated the presence of a conserved element we call the ARS motif. The ARS motif in CD45 exons 4–6 consists of the sequence MCYYGCGAGCA ("long repeat") in tandem with a shorter copy (MCYGGCA), in which M represents cytosine or adenine and Y is any pyrimidine (19). In all three CD45 variable exons, mutation of the GCA core of the ARS repeats abolishes activation-induced exon repression; however, only in exon 4 has the ARS motif been determined to be the sole activation-sensitive regulatory element (19).

To confirm the importance of the ARS consensus motif in exons 5 and 6 and determine whether these exons contain additional sequences that mediate their observed pattern of expression in T cells, we examined the splicing of exons 5 and 6 using chimeric minigenes expressed in T cell-derived JSL1 cells. We have previously shown that the JSL1 cell line recapitulates all aspects of CD45 alternative splicing (10); thus,
these cells provide a useful background for identifying sequences that regulate exon splicing in resting and activated cells. We first sought to confirm whether or not the splicing of CD45 exons 5 and 6 was in fact regulated upon T cell activation and whether this regulation was inherent to the individual exons. When CD45 exon 5 and a few hundred nucleotides of flanking intron are fused directly between CD45 constitutive exons 3 and 7, we indeed observe a pattern of exon 5 expression consistent with that observed in the endogenous gene (Fig. 1B, CDS). RT-PCR analysis of minigene-derived mRNA reveals almost complete inclusion of exon 5 in resting cells but partial repression of this exon upon activation. For this minigene and all others in this study, we quantitate the change in isoform expression upon treatment with PMA by calculating a -fold repression (FR) value. FR is equal to the change in the amount of three-exon product observed for each minigene upon PMA stimulation of cells. -Fold repression values shown were calculated as described in the legend to Fig. 18. For C and D, the bars marked by double asterisks were significantly different from the wild-type SC5 control (p < 0.001) as detailed in supplemental Table I.

To further delineate the sequences most relevant to exon expression, we next engineered minigenes that contain the bulk of sequences internal to exons 5 or 6, cloned into an unregulated minigene derived from the human β-globin gene (gray gradient). The locations of substitutions relative to the start of exon 5 are given for each mutant (m1–m6). B, RT-PCR analysis of minigene-derived RNA as described in the legend to Fig. 1. C, quantitation of percentage of basal inclusion (i.e. percentage of 3E product) in resting cells for each minigene. Values shown are derived from at least four independent clones assayed in at least two separate experiments. D, -fold repression observed for each minigene upon PMA stimulation of cells. -Fold repression values shown were calculated as described in the legend to Fig. 18. For C and D, the bars marked by double asterisks were significantly different from the wild-type SC5 control (p < 0.001) as detailed in supplemental Table I.

FIGURE 2. CD45 exon 5 contains multiple sequences that influence its pattern of inclusion. A, a schematic of block scanning mutagenesis of exon 5 in the context of the SC5 minigene, which contains all of CD45 exon 5 and surrounding intron (light gray) flanked by intron and exon sequences from the human β-globin gene (gray gradient). The locations of substitutions relative to the start of exon 5 are given for each mutant (m1–m6). B, RT-PCR analysis of minigene-derived RNA as described in the legend to Fig. 1. C, quantitation of percentage of basal inclusion (i.e. percentage of 3E product) in resting cells for each minigene. Values shown are derived from at least four independent clones assayed in at least two separate experiments. D, -fold repression observed for each minigene upon PMA stimulation of cells. -Fold repression values shown were calculated as described in the legend to Fig. 18. For C and D, the bars marked by double asterisks were significantly different from the wild-type SC5 control (p < 0.001) as detailed in supplemental Table I.

Regulation of CD45 Exons 5 and 6

To identify individual ESS or ESE sequences within CD45 exons 5 and 6, we next undertook a systematic mutagenesis of each of these exons (Figs. 2 and 3). We replaced regions of 15 (exon 6) or 20 (exon 5) nucleotides of exonic sequence with heterologous sequence previously shown to have no splicing-regulatory activity (21). For each exon, the substitutions, designated m1–m6 for exon 5 or m1–m9 for exon 6, were generated in the context of a minigene that contained all of exon 5 or exon 6 and a short stretch of flanking intron inserted between two constitutive exons of the β-globin gene (SC5 or SC6; Figs. 2A and 3A). SC5 and SC6 are identical to constructs studied previously (19) and were used as a background for the mutagenesis instead of SC-E5 and SC-E6, since we can more readily detect both two-exon and three-exon products from SC5 and SC6, thus allowing more accurate comparison between wild type and the mutants.

For exon 5, our mutagenesis scan reveals three primary regions of interest (Fig. 2). Mutation of region 3 results in an almost complete switch from three-exon to two-exon product in resting cells (m3; Fig. 2, B and C), although this mutation has no effect on the ability of PMA to induce further exon repression (m3; Fig. 2, B and D). Hence, the effect of the m3 mutation suggests that region 3 may function as a basal ESE (see Fig. 4). In contrast, mutation of region 2 has no consequence on exon inclusion in resting cells but renders the splicing entirely resistant to PMA stimulation (m2; Fig. 2, B–D). Importantly, region 2 contains both of the ARS motif repeats in exon 5 (see Fig. 5D). Thus, the effect shown here of substitution m2 is consistent with the splicing phenotype observed previously upon the introduction of point mutations within the ARS motif of exon 5 (19). Substitution of region 1 also causes a slight reduction in the PMA-induced -fold repression of exon 5, most likely due to disruption of the sequence context immediately adjacent to the ARS motif. However, beyond m2 and m3, the only other mutation that significantly alters the splicing pattern of exon 5 is substitution of region 4 (m4). Exon 5 is included in all of the transcripts derived from the m4 minigene, implicating region 4 as a sequence that normally functions to partially repress exon inclusion (Fig. 2B and C). Since inclusion of exon 5 is absolute in m4-derived mRNAs, we cannot meaningfully quantitate a -fold repression value for this minigene (Fig. 2D). Therefore, we cannot differentiate between a direct role of region 4 in activation-induced exon repression versus region 4 acting to keep the overall level of splicing sufficiently suboptimal to render it sensitive to activation-induced repression via region 2.
Regulation of CD45 Exons 5 and 6

CD45 exon 6 also contains multiple splicing-regulatory sequences as determined by scanning mutagenesis (Fig. 3). The largest and most significant effects on the basal level of exon 6 splicing are observed upon mutation of regions 1, 5, and 7 (m1, m5, and m7; Fig. 3, B and C).}

Virtually all of the mRNA derived from the m1 minigene exclude exon 6, whereas inclusion of exon 6 is the predominant pattern for minigenes m5 and m7. Interestingly, whereas both regions 5 and 7 contain homology to the ARS consensus, region 7 contains the longer ARS motif sequence and is the only sequence within exon 6 absolutely required for PMA-induced alternative splicing (FR = 1; Fig. 3D). Whereas we do observe a partial decrease in PMA sensitivity with the exon 6 minigene m1, this is most likely due to a limitation in how much more exon 6 can be repressed in this construct beyond that already occurring in resting cells.

The scanning mutagenesis is indispensable for identifying sequences necessary for the splicing pattern of exons 5 and 6, but it does not confirm that particular sequences are bona fide ESE or ESS sequences sufficient to induce exon inclusion or silencing. The mutagenesis shown in Figs. 2 and 3 suggests that region 3 of exon 5 (E5:R3) and region 1 of exon 6 (E6:R1) may function as ESEs, since mutation of both of these regions decreases exon inclusion. Moreover, the sequence encompassed by E6:R1 is highly reminiscent of the well characterized purine-rich ESE motif (see Fig. 4B). To test whether E6:R1 or E5:R3 could function as a bona fide ESEs, we inserted single or dimeric copies of these sequences within a version of the β-globin minigene that contains a restriction site to facilitate cloning (SC-glo). As shown in Fig. 4, we find that both E5:R3 (Fig. 4A) and E6:R1 (Fig. 4B) function in a dose-dependent manner to increase exon inclusion, confirming their identity as separable ESE-regulatory elements. Interestingly, E5:R3 is a particularly potent splicing enhancer despite having little, if any, sequence homology to previously characterized ESE motifs.

The scanning mutagenesis in Figs. 2 and 3 also highlights sequences within exons 5 and 6 that are potential basal ESS candidates, since their integrity within exons 5 and 6 serves to repress inclusion of these exons in resting cells. Within exon 6, regions 5 and 7 (E6:R5 and E6:R7) are both predicted to function as ESSs, as is region 4 of exon 5 (E4:R5). Consistent with the scanning mutagenesis results, both E6:R5 and E6:R7 are sufficient to induce exon skipping in a dose-dependent manner when inserted into the SC-glo background (Fig. 5, A and B; p < 0.001 for difference between single and double copies; see supplemental Table I).
GCA core of the ARS repeats in exons 4 and 6 not only leads to a loss of activation responsiveness but also results in a dramatic increase in basal exon inclusion (19). The one observation inconsistent with a role of the ARS motif in basal exon repression is the observation that substitution or mutation of the ARS-containing region 2 within exon 5 (E5:R2) does not significantly increase exon inclusion in resting cells (m2; see Fig. 2 and Ref. 19). However, we do find that insertion of a single or dimeric copy of the E5:R2 sequence into the SC-glo background is sufficient to promote basal exon skipping (Fig. 5D; \( p < 0.001 \) and 0.01, respectively). Therefore, we conclude that the ARS sequence does contribute to basal exon silencing, although this activity is somewhat context-dependent.

We also note that the individual ARS-containing regions (E5:R2, E5:R4, E6:R5 and E6:R7) are not sufficient to mediate significant activation-induced exon repression, since the -fold repression values for all constructs shown in Fig. 5 are less than 1.6 (see Fig. 5 legend). In our studies with CD45 exon 4, we have found that robust activation-induced exon repression requires a 60-nucleotide sequence encompassing the ARS repeats, although ARS repeats themselves are the only specific sequences that are absolutely essential for this activity (19). Therefore, we wanted to identify the minimal sequence from exons 5 and 6 that is sufficient for activation-induced exon repression and confirm the requirement for the ARS repeats in this minimal context.

Similar to our observations with CD45 exon 4, a 60-nucleotide sequence from exon 6 spanning regions 5–8 is sufficient to mediate activation-induced exon repression of a heterologous unregulated exon (Fig. 6A, E6:R5–8). The PMA-induced -fold repression of E6:R5–8 is comparable with that observed for the wild-type exon 6 (see CD6; Fig. 1B). The addition of further exon 6 sequences to the E6:R5–8 construct did not increase PMA-induced exon skipping, whereas reduction in the amount of exon 6 sequence to regions 6–8 or regions 5–7 diminished PMA-induced exon repression (data not shown). Importantly, the 60-nucleotide element encompassed by regions 5–8 contains all of the ARS motif elements from CD45 exon 6. Moreover, mutation of the ARS core GCA sequences within E6:R5–8 eliminates both basal and activation-induced repression (Fig. 6A, E6:R5–8mARS). In contrast to what we observe with CD45 exons 4 and 6, virtually the entire sequence of CD45 exon 5 is required to confer activation-induced repression on a heterologous exon (Fig. 6B, E5:R1–5). However, point mutations within the ARS core completely abolish PMA-induced skipping of the exon 5 chimeric minigene (Fig. 6B, E5:R1–5mARS). This requirement for the ARS motif within the minimal regulatory elements of exons 5 and 6 is consistent with what has been observed for the wild-type exons 4–6 (19) and demonstrates the central role of the ARS motif in activation-induced exon skipping.

Recently, we have identified the heterogeneous ribonucleoprotein hnRNP L as the primary protein that associates with the ARS repeats within CD45 exon 4 and mediates repression of this exon under both rested and activated conditions (20). To determine whether hnRNP L also binds the minimal activation-responsive sequence from CD45 exons 5 and 6 in an ARS-dependent manner, we synthesized \(^{32}P\)-labeled RNA corresponding to the 60-nucleotide E6:R5–8 sequence or the 100-nucleotide E5:R1–5 sequence by \textit{in vitro} transcription. These RNAs were incubated under splicing conditions with nuclear extract derived from JSL1 cells, and proteins that associate with E6:R5–8 or E5:R1–5 were covalently cross-linked to the RNA by treatment with UV light. Following digestion of the RNA, proteins bound to the RNA remain covalently attached to \(^{32}P\)-labeled nucleotides, such that they can be

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**FIGURE 5.** CD45 exon 6 contains potent ESS sequences, whereas ESS activity from exon 5 is less robust. RT-PCR analysis of minigene-derived RNAs as described in the legend to Fig. 1. A single or double copy of regions from exons 5 and 6 was inserted into a \( \beta \)-globin minigene (SC-glo). The percentage of two-exon product (i.e. percentage of exon skipping) is shown for each minigene under resting conditions. Percentage of exon skipping values are the average of at least four independent clones assayed in at least two separate experiments. Inserted sequences are shown in each panel with the short and long versions of the ARS motif indicated by a solid underline and the partial match to the short ARS motif denoted by a dashed underline. Constructs and statistics for each panel are as follows. A, insertion of region 5 of exon 6 (E6:R5). No significant effect of stimulation was observed for any of the minigenes (FR < 1.1 ± 0.25). B, insertion of region 7 of exon 6 (E6:R7). No significant effect of stimulation was observed for any of the minigenes (FR < 1.6 ± 0.2). C, insertion of region 4 of exon 5 (E5:R4). -Fold repression for all constructs was <1.3 ± 0.5. D, insertion of region 2 of exon 5 (E5:R2). -Fold repression for all constructs was <1.0 ± 0.3. For all constructs in A–D, S.D. (shown) and \( p \) values (supplemental Table I) were also calculated.

However, the inherent silencing activity of E5:R4 is marginal, since no silencing is observed with a single copy of E5:R4, and only a modest increase in exon repression is observed in the presence of two copies of E5:R4 (Fig. 5C; \( p = 0.026 \) for E5:R4 × 2 versus E5:R4). Regions E6:R7 and E6:R5 contain the long (MCYYGCAMGCA) and short (MCYYGCA) components of the ARS motif respectively, whereas E5:R4 contains only an imperfect match to the short ARS motif (see underlines in Fig. 5). Thus, it appears that the strength of basal exon repression may correlate with homology to the ARS consensus sequence. A role of the ARS consensus sequence in basal exon silencing is consistent with our previous observation that mutation of the

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visualized following resolution on an SDS-polyacrylamide gel. As shown in Fig. 6, C and D, autoradiography of SDS-polyacrylamide gels following the UV cross-linking assay reveal that both E6:R5–8 and E5:R1–5 bind to four prominent proteins that migrate at ~75, 55, 38, and 30 kDa. Strikingly, only the binding of the 75-kDa protein is reduced by mutations in the ARS repeats (Fig. 6, C [E6:R5–8 versus E6:R5–8mARS] and D [E5:R1–5 versus E5:R1–5mARS]). The specificity of the 75-kDa protein for the wild-type ARS sequences was also confirmed by binding...
importantly, the ARS-specific 75-kDa protein bound to both
E5:R1–5 and E6:R5–8 was identified as hnRNP L based on the ability of
the cross-linked protein to be selectively precipitated by antibodies
specific for hnRNP L but not another hnRNP family member, hnRNP U
(Fig. 6, C and D, right panels). Antibodies specific for another hnRNP
protein, PTB, precipitate the 55-kDa protein bound to E6:R5–8 or
E5:R1–5. The binding of PTB to exons 5 and 6 in an ARS-independent
manner is consistent with our previous observation that the ESS1 of
CD45 exon 4 binds to PTB in addition to hnRNP L but that the associ-
sequences in the regulation of CD45 isoform expression. In particular, the relative strengths of the regulatory sequences that we have characterized in each of the CD45 variable exons are consistent with the observed physiological expression of the CD45 protein. The ESS activities within exons 4 and 6 are highly robust, whereas exon 5 has a relatively weak ESS but a particularly strong ESE. This difference in the balance of regulatory pressures provides a mechanistic explanation for the observation that exon 5 is included in the vast majority of transcripts produced in resting cells and even many of the transcripts expressed following activation, whereas inclusion of exons 4 and 6 is much less frequent (see Fig. 1).

Finally, in addition to explaining the normal physiologic expression of CD45, a detailed understanding of the splicing-regulatory elements is critical for interpreting the biological effects of polymorphisms and mutations within the CD45 gene. At least two polymorphisms in CD45 exon 4 and one in CD45 exon 6 have already been described in the human population to alter CD45 isoform expression and have been potentially linked to autoimmune disease susceptibility (13–15, 22–24). These three polymorphisms all fall within or near the regulatory sequences described here or in our previous study (18). Given the importance of the splicing-regulatory sequences within CD45 exons 4–6, we predict that many more mutations within the CD45 gene could exist that alter protein expression and immune function. Moreover, our data suggest that mutations within the hnRNP L protein might also influence CD45 isoform expression. Therefore, the data presented here provide a framework for predicting and understanding the effect of human mutations and polymorphisms that may be found in CD45 and/or alter isoform expression of the CD45 protein tyrosine phosphatase.

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