Mammalian Genome 10, 95–101 (1999).

Mammalian Genome
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Genomic sequence comparison of the human and mouse adenosine deaminase gene regions

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Received: 27 March 1998 / Accepted: 22 September 1998

Abstract. A challenge for mammalian genetics is the recognition of critical regulatory regions in primary gene sequence. One approach to this problem is to compare sequences from genes exhibiting highly conserved expression patterns in disparate organisms. Previous transgenic and transfection analyses defined conserved regulatory domains in the mouse and human adenosine deaminase (ADA) genes. We have thus attempted to identify regions with comparable similarity levels potentially indicative of critical ADA regulatory regions. On the basis of aligned regions of the mouse and human ADA gene, using a 24-bp window, we find that similarity overall (67.7%) and throughout the noncoding sequences (67.1%) is markedly lower than that of the coding regions (81%). This low overall similarity facilitated recognition of more highly conserved regions. In addition to the highly conserved exons, ten noncoding regions >100 bp in length displayed >70% sequence similarity. Most of these contained numerous 24-bp windows with much higher levels of similarity. A number of these regions, including the promoter and the thymic enhancer, were more similar than several exons. A third block, located near the thymic enhancer but just outside of a minimally defined locus control region, exhibited stronger similarity than the promoter or thymic enhancer. In contrast, only fragmentary similarity was exhibited in a region that harbors a strong duodenal enhancer in the human gene. These studies show that comparative sequence analysis can be a powerful tool for identifying conserved regulatory domains, but that some conserved sequences may not be detected by certain functional analyses as transgenic mice.

Introduction

Large-scale comparisons of the genes of humans and disparate species provide insights into gene structure, function, and evolution not easily obtained from the analysis of human sequences alone. Combinatorial complexity inherent in multifactor binding site determination of enhancers and promoters (reviewed in Arnone and Davidson 1997) ensures that sequence-based recognition of regulatory regions will remain a difficult challenge. Rodent species generally provide a well-characterized, practical model for testing hypotheses that may result from large-scale comparisons (Koop 1995). Their genetic similarity to humans should facilitate identification of homologous loci, yet their evolutionary distance from us should permit conserved and nonconserved sequences within a locus to be distinguished (Hood et al. 1992). However, only eight human–rodent comparisons >20 kb have been published, as few genes have been sequenced from more than one species. Five of these are in clustered multigene families: the immunoglobulin heavy chain J_H-Cμ-Cδ region (Koop et al. 1996), the T-cell receptor Cα/Cδ region (Koop et al. 1994; Koop and Hood 1994), the β-globin gene cluster (reviewed in Hardison and Miller 1993; Hardison et al. 1994), the α- and β-myosin heavy chain genes (Liew et al. 1990; Epp et al. 1993, 1995; GenBank r84, Koop 1994), and the γ-crystallin gene cluster (den Dunnen et al. 1989). The remaining large human–rodent comparisons are from two DNA repair genes, XRCC1 and ERCC2 (Lamerdin et al. 1995, 1996) and the Bruton's tyrosine kinase locus (Oeltjen et al. 1997).

ADA is a key enzyme of purine metabolism that catalyzes the deamination of adenosine or deoxyadenosine. Its deficiency in humans results in an accumulation of metabolites toxic to lymphocytes, causing severe combined immunodeficiency (SCID; Giblett et al. 1972). ADA-deficient homozygous mice die perinatally owing to disturbed purine metabolism, severe liver cell impairment, epithelial cell death in the small intestine, and atelectasis (Migchielsen et al. 1995; Wakamiya et al. 1995). Although expressed ubiquitously in mammals, ADA levels vary widely according to cell type, species, and stage of development or differentiation (Witte et al. 1991; Chinsky et al. 1990). Its expression profile thus differs somewhat from that of most mammalian genes, which are usually expressed either ubiquitously at relatively low levels or in a restricted set of cell types at moderate to high levels. In human tissues, expression varies over a 1000-fold range, with highest levels in thymus and duodenum (Aronow et al. 1989). In mice, the highest levels are in tongue, esophagus, forestomach, maternal decidua, and fetal placenta, as well as in thymus and duodenum (Knudsen et al. 1988; Chinsky et al. 1990; Mohammedeli et al. 1993). ADA expression in human cells is regulated mainly at the level of transcription initiation (Lattier et al. 1989), but in some cell types transcriptional arrest may play a regulatory role (Chen et al. 1990).

Modular organization of *cis*-regulatory elements has been observed in a number of genes, especially those expressed in complex temporal and/or spatial patterns (reviewed in Kirchhamer et al. 1996). Mounting evidence indicates that distinct regulatory modules also govern the diverse expression pattern of human and mouse ADA. A GC-rich TATAAA-box-deficient promoter is required for basal transcription in both species (Valerio et al. 1985; Ingolia et al. 1986; Rauth et al. 1990; Innis et al. 1991; Dusing and Wiginton 1994). T-cell-specific expression of ADA is primarily regulated by a potent enhancer identified and characterized in human intron 1 (Aronow et al. 1989, 1992) and subsequently confirmed in mouse intron 1 (Brickner et al. 1995; Winston et al. 1995, 1996). Sequences flanking the human thymic enhancer are required for position-independent, copy-proportional thymic expression in transgenic mice, but not in transient transfection of human T-cell lines. These elements, termed facilitators, are apparently necessary in establishing a proper chromatin configuration for thymic enhancer function (Aronow et al. 1992, 1995). A 3.3-kb

tionally activated basal levels of chloramphenicol acetyltransferase (CAT) reporter expression in all transgenic mouse tissues tested; this ubiquitously activating element was found to be separable from the thymic enhancer (Winston et al. 1996). Similar ubiquitous activation was also shown in the 2.3-kb fragment harboring the human thymic enhancer and facilitators (Aronow et al. 1989). Recent studies in our laboratory have identified a 3.4-kb human ADA gene segment centered in intron 2 capable of driving high-level CAT expression in transgenic mouse duodenal epithelium (Dusing et al. 1997). Functional characterization of this element is ongoing. Separate segments within 6.5 kb of mouse ADA gene 5′ flanking sequences regulate expression postnatally in the forestomach and prenatally in the placenta (Winston et al. 1992, 1995). This upstream fragment was also used to drive placental expression from an ADA minigene construction that rescued homozygous ADA-deficient mice from perinatal lethality (Blackburn et al. 1995). However, ADA expression in rescued mice was limited to the gastrointestinal tract, primarily the forestomach, and was accompanied by lymphoid-specific metabolic disturbances (Blackburn et al. 1996). Modules regulating high-level expression in other tissues such as maternal decidua and mouse duodenum, tongue, and esophagus have yet to be identified. ADA-deficient mice rescued by introduction of a human ADA gene-containing transgene into mouse zygotes reflected a human expression pattern, expressing only low-level human ADA in the upper alimentary tract, as opposed to high-level endogenous mouse ADA. Thus, the human gene apparently lacks regulatory elements necessary for high-level ADA expression in the mouse upper alimentary tract (Migchielsen et al. 1996).

In this study, we have attempted to identify conserved regions, particularly in noncoding sequence, that correlate with ADA gene segments that may play important functional or structural roles. Despite low-level similarity throughout the sequence compared, we identified several distinct, highly conserved blocks of noncoding sequence. Two, the promoter and thymic enhancer, have previously demonstrated functional significance. Several conserved regions were observed to which no function has yet been ascribed, as well as at least one functional region that is apparently not well preserved between mouse and human.

Materials and methods

Analysis of human and mouse ADA sequences. General patterns of similarity between the human and mouse ADA sequences were initially plotted on x and y axes by dot matrix analyses as implemented by Inherit GeneAssist (v1.1, Perkin-Elmer/ABD, Foster City, Calif.) and Dotter (Sonnhammer and Durbin 1995). In GeneAssist, plotted dots represent 16 matches in a window of 20 bp. Adjacent windows overlapped by 10 bp. Diagonal lines reflect colinear dots of similarity between the two sequences. Parameters chosen for presentation showed a moderate background of dots with regions of similarity clearly indicated. A global alignment of these sequences was determined with the MAP program (Huang 1994). Parameters used to determine the alignment were: match = 10, mismatch penalty = −9, gap open penalty = 27, gap extend penalty = 10 (to a maximum of 10 base positions). Of the range of parameters examined, the values used in the final alignment attempted to minimize the number of gaps and maximize overall similarity. Thus, the overall similarity value of aligned regions may be overestimated. A plot of local similarity was based on overlapping 24-bp windows where gaps were counted 1 difference over 1 position, irrespective of gap length (window overlap was 12 bp).

Results

This study arose as an extension of our previous comparison of the mouse and human ADA gene thymic enhancers (Brickner et al. 1995). We initially sequenced a 3.3-kb mouse intron 1 fragment (GenBank U72392) containing the thymic enhancer for comparison with the human sequence (GenBank M13798). Subsequent determination of the mouse ADA gene sequence by Rodney Kellems' laboratory (GenBank U73107) has resulted in the more complete comparison reported here.

The ADA gene spans ~23 kb in mouse and ~32 kb in humans. The human and mouse genes are similarly organized, contain 12 exons and 11 introns, and encode proteins of 363 and 352 amino acids, respectively, which are 83% identical (Wiginton et al. 1984; Yeung et al. 1985). Mouse ADA lacks the 11 C-terminal amino acid residues of human ADA owing to an additional stop codon in exon 11 (Al-Ubaidi et al. 1990). Murine ADAs 1056-bp open reading frame shares 81% sequence similarity with its human counterpart.

Figure 1 depicts the human and mouse ADA gene maps, showing the similar organization of the genes (top) and a dot matrix overview of similarity between the genes (bottom). Lengths of exons and the smaller introns are highly conserved. Intron 1 comprises nearly half the length of either gene. Alu repeats in the human gene account for most interspecies differences in intronic size, especially in introns 1, 2, and 6. Within the human gene, 23 Alu repeats were identified (Wiginton et al. 1986), which comprise 18% of the total sequence at a density of 0.62 Alu/kb, exceeding the average density (0.25 per kb) predicted for the rest of the genome (Moyzis et al. 1989).

In the dot matrix comparison in Fig. 1 (bottom), a strong diagonal occurs 3′ of exon 4, a region containing closely spaced exons and small, well-conserved introns. The 3′ half of intron 1 and most of intron 2 comprise a large region of low similarity. The insertion of ten Alu repeats over ~13 kb of human sequence contributes to the discontinuity of the diagonal in this region. Similarity 5′ of the promoter is also low, mainly owing to two nearly contiguous Alu repeats and a hybrid O-type/Alu repeat in the human sequence (Wiginton et al. 1986). Low similarity 3′ of exon 1 results from five Alu repeats in the human sequence as well as two B-type repeats in the mouse sequence (GenBank U73107). Conserved noncoding sequences in Fig. 1 include the promoter region, the thymic enhancer, and an adjacent region just 3′ within intron 1, and a region central to intron 3.

A local alignment was constructed from the human and mouse ADA sequences (data not shown; alignment provided upon request) to more precisely determine the degree of significant sequence conservation. On the basis of 24-bp windows and 12-bp overlapping window shifts, local levels of similarity were determined and plotted (Fig. 2A) across the entire aligned sequence. As indicated by the dashed lines in the top map in Fig. 1, the mouse gene contains about 1.9 kb and 1.2 kb of 5′ and 3′ flanking sequence, respectively, extending beyond the alignment with the human gene.

A critical parameter to establish in the sequence comparison of two species is the level of similarity due to random incorporation of mutations in nontranscribed DNA. Determination of this level facilitates identification of regions actively conserved by forces such as natural selection (Koop 1994). Our comparison revealed an average level of 67.7% sequence similarity over the ADA gene region, based on aligned regions only. Since a basal divergence level between mouse and human noncoding DNA sequences has not been established, we utilized this 67.7% value (indicated as a solid line in Fig. 2A) as a background similarity level in assigning significance to high similarity. Significant conservation in Fig. 2 is indicated not only by high percentage similarity in a given window (height of peak), but also by extended similarity over consecutive windows (width of peak). Large gaps in the plot indicate consecutive windows in which alignment scores fail to exceed 50% similarity or sequence is not present in one of the two sequences compared. These gaps often indicate the presence of repetitive elements, depicted by arrows beneath the plot, but some sequences could be neither aligned nor identified as repetitive DNA. To facilitate recognition of conserved regions, we adjusted the align-

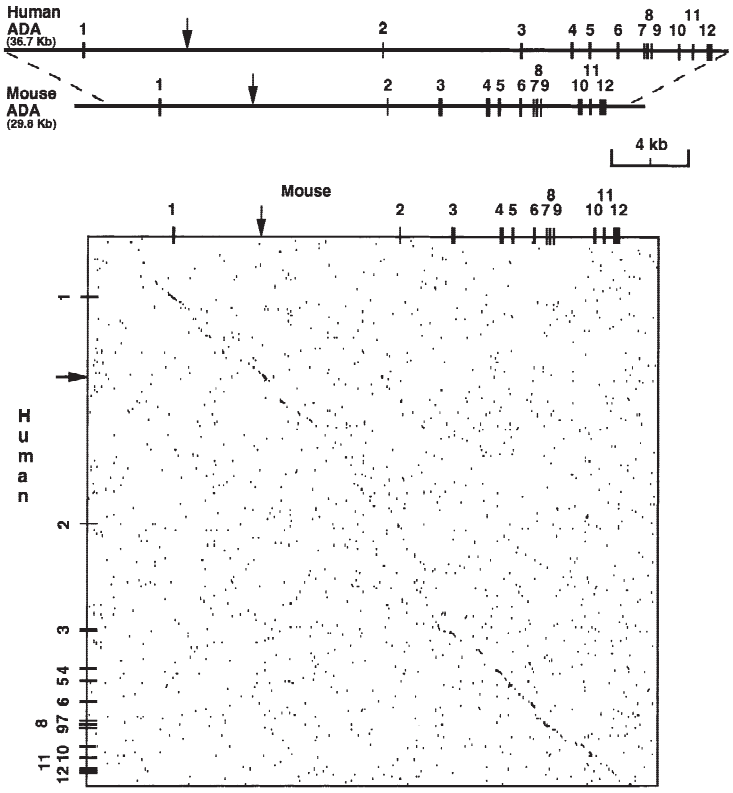

Fig. 1. Top, exonic map of human (GenBank M13792) and mouse (GenBank U73107) ADA genes. Dashed lines indicate boundaries of alignment; vertical bars, positions of exons; arrows, thymic enhancer region. Bottom, overall gene dot matrix analysis. Each dot indicates a ≥16 bp match in a 20-bp window. Windows are moved to adjacent positions by incrementing window size times 0.5. Dots forming diagonal lines represent extended areas of similarity.

76.6%, the mean overall similarity plus one standard deviation (SD = 8.9%). The plots clearly illustrate a set of distinctly conserved regions.

Conservation of the ADA coding sequence is obvious in Fig. 2 and is substantially higher (81%) than the overall similarity of 67.7%. Similarity throughout the aligned noncoding sequence (67.1%) is only slightly lower than the 67.7% overall similarity, since coding sequences comprise only a small portion (<3.5%) of either gene. Exons 4 and 5 are especially conserved, the latter containing only three mismatches over 41 bp. Twelve segments exceeded 90% similarity over at least 24 bp, eight of which correspond to exons 2 through 6, 8, 9, and 10. The remaining four lie in noncoding regions (see below). Murine exon 12 contains only untranslated and polyadenylation sequences (Al Ubaidi et al. 1990) and is not highly similar to human exon 12, which contains the final three amino acid codons and 3′ untranslated region (Wiginton et al. 1986).

Several noncoding areas in the alignment plots exhibit extended similarity and coincide with those observed in Fig. 1. Two correspond to known functional domains shared by mice and humans, the promoter and thymic enhancer. Our previous comparison of the human and murine thymic enhancer regions indicated 71.1% similarity overall, four subregions with ≥80% similarity over ≥24 bp, and several conserved transcription factor consensus sequences within these subregions (Brickner et al. 1995). We subsequently determined similarity in this region over a wider window, as shown in Fig. 3. In a comparison of 1.1 kb of mouse ADA promoter/exon 1 sequence to its human counterpart, Al-Ubaidi et al. (1990) identified 10 domains exhibiting ≥85% similarity over ≥15 bp. In this previous comparison, Domains I–IV, which encompass the proximal promoter region through the first 69 bp of mouse intron 1, exhibit similarity (73.8% over 233 bp) very analogous to that observed in the thymic enhancer despite the inclusion of 33 bp of coding sequence. Domains from this previous comparison which lie further 5′ of the transcription initiation site exhibit less extensive similarity. The extensive similarity (>70% over

the idea of using comparative analysis to identify conserved regulatory regions within ADA noncoding sequence. Comparable levels of noncoding sequence similarity for a functional regulatory element (70% over 370 bp) were established in a comparison of human and mouse T-cell-specific enhancers of the T-cell receptor Cδ gene (Koop et al. 1992). A placenta-specific regulatory element identified in the mouse ADA gene (Shi et al. 1997) lies 5′ of the mouse sequence used in this study. Since the analogous region of the human gene has not been sequenced, we could not evaluate the conservation of this element. Comparison of the mouse placental enhancer sequence to the entire known human ADA sequence yielded no significant similarities (data not shown).

Since the promoter and thymic enhancer are clearly evident in Figs. 1 and 2, we attempted to detect additional conserved elements having potential functionality. Regions of extended similarity in Fig. 2B were tested by utilizing the alignment to determine whether their similarities exceeded >70% over >100 bp of human sequence. This level was chosen to permit detection of regions with similarities comparable to, yet less extensive than that exhibited by the thymic enhancer and promoter regions. Table 1 summarizes the lengths, similarity, and positions of 19 conserved regions (CRs) that meet these criteria. All CRs contain multiple 24-bp regions with much higher levels of similarity than 70%. Most CRs have spikes in Fig. 2 that exceed 2 SD (85.5%). CRs containing exons include conserved adjacent sequence, some of which is associated with consensus splicing signals. All exons but exon 12 exhibited enough similarity for inclusion as a CR. Exons 7 and 8, sequences flanking these two exons, and intron 7 were grouped as CR13 according to our criteria.

CRs in noncoding sequence in Fig. 2 are also presented in Table 1. Some, particularly CRs 3 and 4, are conserved over regions as extensive as those containing the exons. The promoter/exon1 (CR1a) and thymic enhancer regions (CR3) are obvious in Fig. 2B. Since coding sequence from exon 1 comprises only about 14% (33 bp) of CR1a, we designated as it a noncoding CR. CRs 1a and 1b are separated by two copies of a 31-bp direct repeat in the

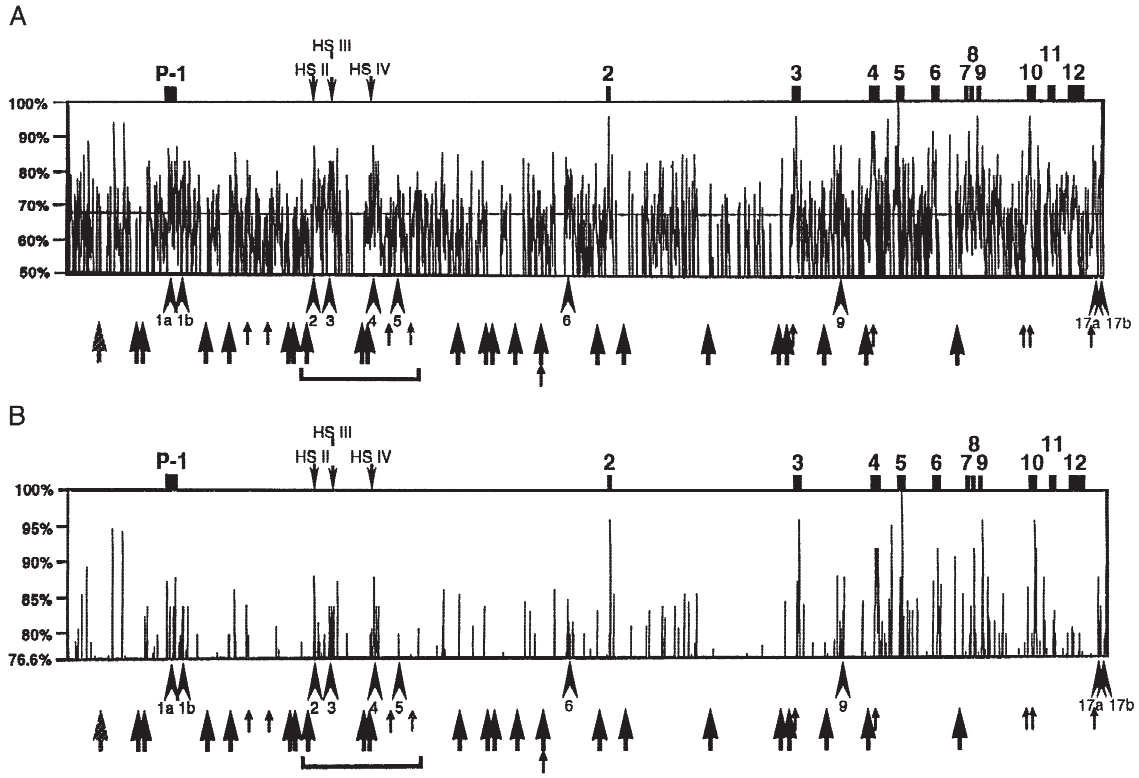

Fig. 2. Human and mouse ADA gene local similarity analysis. An alignment was constructed from the human and mouse ADA sequences. Local levels of sequence similarity were calculated from a 24-bp window that shifted along the alignment in overlapping 12-bp intervals. Level of similarity (vertical axis) is plotted against alignment position (horizontal axis). Positions of exons and three HS within intron 1 (see text) are indicated above graph, as well as the promoter/exon 1 region, designated as P-1. **(A)** Average similarity between human and mouse aligned sequences, indicated by solid line, is 67.7% (gap counted as 1 difference over 1 alignment position). Large arrows below graph indicate positions of Alu repeats in human sequence; small arrows indicate positions of B1 and B2 repeats in mouse. Large and small stippled arrows indicate the positions of a hybrid O-type/Alu repeat in the human sequence (Wiginton et al. 1986) and a repeat region similar to an unidentified repeat in the Chinese hamster rhodopsin gene locus (Gale et al. 1992), respectively. Bracket below CR2 through CR5 indicates region included in the mouse/human comparison from our initial 3.3 kb of mouse intron 1 sequence, represented in greater detail in Fig. 3. **(B)** Graph emphasizing regions of high similarity. Graph identical in all respects to Fig. 2A except that minimum value on vertical axis equals average similarity plus one standard deviation (76.6%, SD = 8.9%). CRs (see text and Table 1) within noncoding sequence are indicated by arrowheads below the graph.

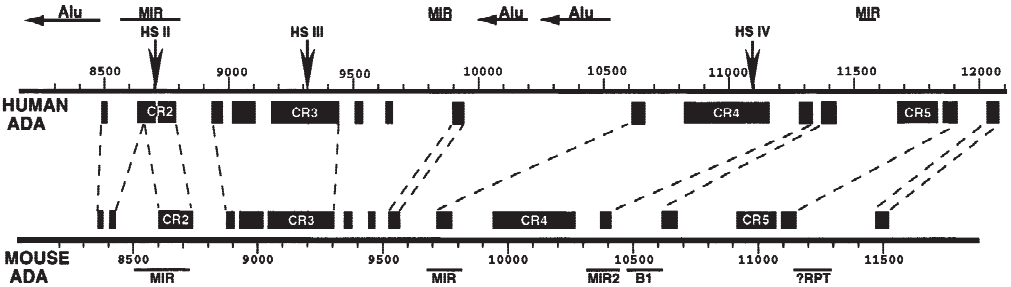

Fig. 3. Detailed map of high similarity in intron 1 corresponding to bracketed region in Fig. 2. Sequence from a 3.3-kb mouse ADA intron 1 fragment (GenBank U72392; numbered according to GenBank U73107) was compared with the analogous human ADA sequence (GenBank M13792). Black rectangles represent blocks of high similarity (≥70% over ≥20 bp of human sequence); CRs are labeled accordingly. Repetitive elements indicated by horizontal arrows or bars above or below maps; ?RPT is repeat region similar to an unidentified repeat in the Chinese hamster rhodopsin gene locus (Gale et al. 1992). Positions of three DNase I HS in human intron 1 (see text) are indicated at top of map. Alignment parameters used are identical to those in Fig. 4.

a single CR. CR4 lies 3′ of the thymic enhancer and exhibits similarity (70.5% over 336 bp of human sequence), more extensive than that of the thymic enhancer and possibly consistent with a functional role (see Fig. 4). Although CR4's function is unknown, it corresponds to the fourth of an array of six thymus-specific DNase I hypersensitive sites (HS) previously identified within human ADA intron 1 (Aronow et al. 1989). HS regions are thought of open chromatin that allow transcription factors access to their binding sites within regulatory regions in the process of functional activation (reviewed in Elgin 1988). The human thymic enhancer segment within CR3 is associated with HS III, the only HS of the six with clear functional significance (Aronow et al. 1992). HS II is also conserved (CR2), but over a markedly shorter span (71.3% over 101 bp). The remaining three HSs in intron 1 were not well

Table 1. Conserved regions identified in a comparison of the mouse and human ADA gene regions.

CR	Length (bp)	% Similarity	Human ADA #s	Mouse ADA #s	Corresponding region
CR1a	233	73.8%	3905–4137	4261–4483	Promoter/exon 1
CR1b	120	70.1%	4269–4388	4686–4808	3′ of exon 1
CR2	101	71.3%	8670–8770	8600–8687	HS II
CR3	284	70.7%	9169–9452	9041–9313	HS III (thymic enhancer)
CR4	336	70.5%	10821–11156	9943–10269	HS IV
CR5	161	72.7%	11674–11834	10825–11002	3′ of HS IV
CR6	108	70.3%	17824–17931	14884–14996	5′ of exon 2
CR7	195	71.3%	19220–19414	16239–16422	Exon 2
CR8	311	72.0%	26318–26627	18977–19274	Exon 3
CR9	104	76.0%	27772–27875	20652–20756	Mid-intron 3
CR10	246	82.5%	28840–29088	21416–21661	Exon 4
CR11	201	80.6%	29745–29945	22032–22228	Exon 5
CR12	331	76.7%	31023–31353	23091–23415	Exon 6
CR13	337	70.3%	32404–32740	23905–24241	Exons 7 and 8
CR14	298	71.5%	32799–33096	24402–24631	Exon 9
CR15	226	72.6%	34345–34537	26222–26418	Exon 10
CR16	141	70.2%	35036–35176	26776–26908	Exon 11
CR17a	250	72.8%	36383–36632	28248–28496	3′ of exon 12
CR17b	104	72.1%	36638–36741	28530–28624	3′ of exon 12

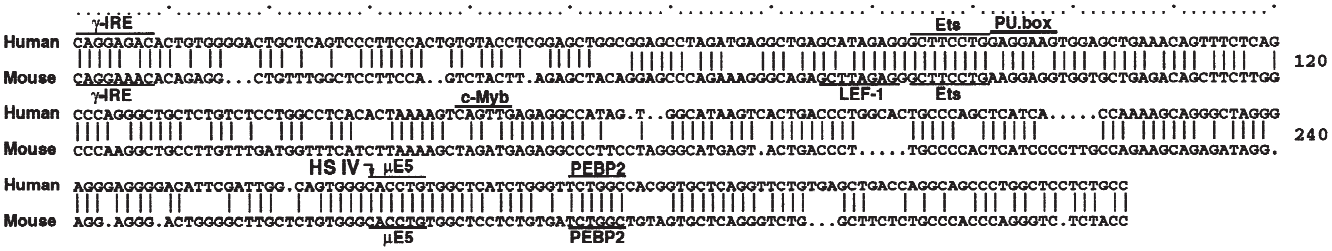

Fig. 4. Sequence alignment of human (top) and mouse (bottom) CR4, the most extensive noncoding CR determined in this comparison. Consensus matches for transcription factor binding sites are shown (overlined, human; underlined, mouse). Alignment corresponds to human bps. 10821–11156 and mouse bps. 9943–10269 from same GenBank accession Nos. as Fig. 3. Alignment parameters are: match = +1; mismatch penalty = 0.9, gap penalty = 1 + 1.7* gap length (to a maximum of 10). **HSIV** = center of HS IV region in human intron 1 (Aronow et al. 1989).

the region of the mouse/human comparison from our initial 3.3 kb of mouse intron 1 sequence (GenBank U72392), shown in greater detail in Fig. 3.

CRs 6, 9, 17a, and 17b also exhibit high similarity in Fig. 2. CR6 lies within a 13-kb human ADA gene segment that drives enhanced duodenal CAT activity in transgenic mice, but lies 5′ of a smaller 3.4-kb segment sufficient for duodenal expression (Dusing et al. 1997). We have yet to establish any relevance of CR6 to aspects of duodenal expression. CR9 lies within intron 3 in both species, and CRs 17a and 17b lie 3′ of the polyadenylation sequences. Interestingly, GenBank U73107 notes that mouse bps 28320–28777, included within CRs 17a and 17b, are similar to GenBank expressed sequence tags (Ests) W71509 and AA031028. CR5 was the only CR detected that did not exhibit obvious conservation in Fig. 2, yet met our criteria for inclusion in Table 1.

In Fig. 2, several short stretches of high similarity in noncoding regions did not meet the length criteria for inclusion as CRs. The 5′-most spike exceeding 90% similarity lies in a region with only four mismatches over 40 bp. The spike between exons 4 and 5 contains 22 bp with 100% similarity. These peaks correspond to bps 1720–1759 and 29500–29521 of the human ADA GenBank sequence, respectively. Some sharp peaks in Fig. 2 may be attributed to alignment artifacts associated with regions adjacent to gaps. Caution must be exercised in interpreting the significance of such conserved short stretches considering the size of the ADA locus. Still, we cannot exclude the possibility that some may have a functional or architectural role. Most of the larger gaps in Fig. 2 represent the insertion of known mammalian short interspersed elements (SINES), which we have identified in the sequences of

Discussion

In order to elucidate the mechanisms of eukaryotic gene regulation, thus furthering fundamental understanding of normal and aberrant biological processes, diverse avenues of investigation must be pursued. Searching for evolutionarily conserved sequences is an approach that could facilitate the identification and characterization of a gene's transcriptional regulatory elements. For instance, one of the first tissue-specific enhancers identified, the immunoglobulin kappa enhancer, was initially noted as a highly conserved region within an intron (Emorine et al. 1983). In this study we have compared 36.7 kb of the human ADA gene with 29.8 kb of its mouse counterpart. We attempted to gain insight into the overall level of conservation, and possible functionality, in this region by comparing the corresponding sequences of the two species.

A mosaic model of genome evolution was proposed by Koop (1995) based on three general patterns of noncoding DNA sequence conservation observed in large (>20 kb) human–rodent sequence comparisons. These consist of a divergent pattern, with high-level sequence similarity limited almost exclusively to the coding regions; a conserved pattern, with high similarity present in both coding and noncoding regions; and a third, mixed pattern of similarity in which conserved and divergent sequences are adjacent to one another within noncoding sequences. These patterns of similarity exhibit fast, slow, and mixed rates of incorporation of mutations, respectively, indicating that different genomic regions evolve at different rates (Koop 1995; Koop et al. 1996). The divergent pattern, initially observed in human–rodent comparisons

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