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ORIGINAL PAPER

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Mapping of the mouse serum amyloid *A* gene cluster by long-range polymerase chain reaction

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Abstract The present study defines the organization of the mouse serum amyloid A (*Saa*) gene cluster on chromosome 7. A polymerase chain reaction (PCR)-based strategy was used successfully to generate a complete map of the mouse *Saa* genes, defining a linkage group of 3′-*Saa2*-5′/5′-*Saa1*-3′/5′-*Saa4*-3′/5′-*Saa5*-3′/5′-*Saa3*-3′, with a maximum size of 45 kilobases (kb). This contrasts with the 150 kb human *SAA* gene cluster, which has been previously defined. The tight linkage of both mouse *Saas* and human *SAAs* is of potential functional significance, since the genes that encode the acute phase serum amyloid A proteins are known to exhibit co-ordinate transcriptional regulation. The present results thus suggest that selective pressure may exist which maintains the co-ordinately transcribed *Saa* genes in close physical proximity. This study, furthermore, demonstrates the utility of a novel PCR-based approach for fine mapping of tightly clustered linkage groups. The strategy used possesses a number of advantages over previously described techniques, such as long-range restriction mapping, since it facilitates the concurrent determination of not only precise relative map positions, but also the relative transcriptional orientations of assayed paired loci. Although presently limited in resolution to genes not more than 27 kb apart, future technical advances are likely to extend the applicability of this approach in mapping experiments to less tightly linked clusters of genes.

Introduction

The serum amyloid A (SAA) proteins are a family of differentially expressed apolipoproteins, some of which are the precursors of amyloid A (AA), the principal component of the amyloid fibrils deposited in reactive secondary amyloidosis (Cohen and Calkins 1959). The SAA proteins, and the genes that encode them, are highly conserved throughout evolution, indicating an important biological function(s) (Uhlar et al. 1994, 1996). Although their principal physiological function has yet to be established, the observation that up to five percent of liver protein synthesis is devoted to the synthesis of SAA proteins during the acute phase response (APR) suggests a central role in the maintenance of homeostasis following inflammatory stimuli (Morrow et al. 1981).

Kisilevsky and Subrahmanyan (1992) presented evidence suggesting that SAA may act as a signal to redirect high-density lipoprotein (HDL) to inflammatory cells, such as macrophages, for the purposes of cholesterol removal during the APR. This hypothesis is strengthened by the observation of altered cholesterol efflux when SAA constitutes more than 50% of the HDL protein (Banka et al. 1995), and, furthermore, by evidence that SAA can serve as a transient cholesterol-binding protein (Liang and Sipe 1995). It has also been suggested that SAA may contribute to wound healing, mediated by collagenase, through a feedback loop system that regulates the extent of tissue degeneration. Rabbit SAA3 has an autocrine collagenase induction activity and, furthermore, acts as a substrate for the induced collagenase (Mitchell et al. 1991, 1993). More recent studies have indicated a potential chemoattractant role for SAA in the recruitment of T lymphocytes, neutrophils, and monocytes into inflammatory lesions (Badolato et al. 1994; Xu et al. 1995). Thus, current evidence suggests that the SAA proteins are likely to be of significant immunological importance [reviewed in Steel and Whitehead (1994)]. It is therefore of considerable interest to further characterize the SAA-encoding genes.

SAA is the product of multiple related genes in several species, the most intensively studied of which are human and mouse. The human *SAA* gene cluster consists of three transcribed genes (*SAA1*, *SAA2*, and *SAA4*) and a pseudogene (*SAA3*), localized to chromosome (Chr) 11p15.1 (Sellar et al. 1994). The mouse *Saa* gene family comprises three acute-phase *Saa (A-Saa)* genes (*Saa1*, *Saa2*, and

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Table 1 Mouse *Saa* locus-specific paired oligonucleotide sequences and size of PCR products in base pairs (bp). Locus-specific oligonucleotides were designed from sequences obtained from the following sources: *Saa1*, *Saa2* and *Saa3* oligonucleotides: Lowell and co-workers (1986b); MSAA5*Eco*RIF, MSAA5LF, and MSAA5LR: de Beer and co-workers (1994); MSAA5LR5′B: A. Butler and A. S. Whitehead, unpublished data

Gene	Oligonucleotide	Sequence	PCR product
Saa1	MSAA1LF	5′-TTGTGTGGGAGTTTCTTACGCCCATAAGCCTTGG-3′	298 bp
	MSAA1LR	5′-TGCCATCACCGTTCTCTGACTGATATTAAATTTC-3′	
Saa2	MSAA2LF	5′-GAATTCTAGAAATGTCAGAACTGTTACACAAAGA-3′	467 bp
	MSAA2LR	5′-CTCCTAGGAAAGCAGTCAGAACTGTTCCTGCAGA-3′	
Saa3	MSAA3LF	5′-GGATCCCATGATTTATCACACATTCATATTTTTC-3′	469 bp
	MSAA3LR	5′-CCATCTAGTGGATTCAGGATAGAGATAGAACATT-3′	
Saa4	MSAA4LF	5′-AGCCTGTGCCTGATACAGTGAATAGGTGTCATTG-3′	265 bp
	MSAA4LR	5′-TCTAAGCTCAAGGAGGTAGACAGAAGACCACACA-3′	
5′-*Saa5*	MSAA5*Eco*RIF	5′-GAAGGTCCTAACGGTCCGAATTCCTCT-3′	222 bp
	MSAA5LR5′B	5′-GCTACTTCCAGGGCAGTGTGGGCTACATAAGACC-3′	
3′-*Saa5*	MSAA5LF	5′-GTCTGCCACTCAGACAGCACTCAGTGGACTAGGC-3′	207 bp
	MSAA5LR	5′-TTATTTTCTGTGATCCATAGCAACTCTTTCTCCC-3′	

Saa3), which are characterized by a rapid and dramatic induction following an inflammatory stimulus, one essentially constitutively expressed gene (*Saa5*), and a pseudogene [(*Saa4*) (Lowell et al. 1986a, 1986b; de Beer et al. 1991, 1994)]. The mouse *A-SAA* genes have been localized to proximal Chr 7, between the pink-eye dilution locus (*p*) and the glucose phosphate isomerase locus [(*Gpi-1*) (Taylor and Rowe 1984; Stubbs et al. 1994)].

We have previously mapped mouse *Saa5* to the interval between the *Cd37* and *Gas2* loci on proximal Chr 7, and demonstrated an *Saa1/Saa2/Saa3/Saa4/Saa5* linkage group (not ordered) with a maximum length of 600 kb (Butler et al. 1995). The present study utilizes the technique of long-range polymerase chain reaction (PCR) to further delineate the organization of the mouse *Saa* gene cluster.

Materials and methods

DNA isolation

High relative molecular mass (M_r) DNA was isolated (Blin and Stafford 1976) from frozen A/J mouse liver (Jackson Laboratories, Bar Harbor, ME).

PCR

Mouse A/J DNA was subjected to PCR using the Expand Long Template PCR System (Boehringer Mannheim, Mannheim, Germany) and *Saa* locus-specific primers for *Saa1*, *Saa2*, *Saa3*, *Saa4*, the 5′ untranslated region (UTR) of *Saa5*, and the 3′ UTR of *Saa5* (Table 1). The mouse *Saa5* gene was sized by PCR using the MSAA5*Eco*RIF oligonucleotide and MSAA5*Eco*RIR (5′-AATTC-GAATTCAATACATAGCCAGGGCTGG-3′), which are complementary to the extreme 5′ and 3′ ends, respectively, of the previously published partial *Saa5* cDNA sequence (de Beer et al. 1994). To ensure sequence specificity, all primer sequences were subjected to BLASTN homology analyses (Altschul et al. 1990), performed at the National Center for Biotechnology Information using the BLAST network service. Expand Long Template PCR conditions were optimized for individual assays, and performed in 0.2 ml thin-walled reaction tubes (Boehringer Mannheim) using an Omnigene temperature cycler (Hybaid, Middlesex, England). PCR products (including appropriate controls) were electrophoresed in 1% agarose (Gibco BRL, Life Technologies, Paisley, England) containing 0.3 ng/μl ethidium bromide (Sigma Chemicals, Dorset, Scotland), alongside 1 kb DNA ladder and high M_r DNA markers (Gibco BRL).

Southern blotting

Following electrophoresis, PCR products were transferred to Hybond-N membranes (Amersham International, Buckinghamshire, England) using a PosiBlot pressure blotter (Stratagene, La Jolla, CA). Filters were baked at 80 °C for 2 h before overnight hybridization, with PCR-generated probes, in 100 mg/ml dextran sulphate, 1% sodium dodecyl sulphate (SDS), 1 M sodium chloride and 1 mg/ml sonicated salmon sperm DNA (Sigma Chemicals) at 65 °C. Hybridized filters were rinsed in 2× standard sodium citrate (SSC) at room temperature, washed for 1 h in 2×SSC, 0.1% SDS at 65 °C and in 0.1×SSC for 30 min at 65 °C before visualization by autoradiography.

Molecular probes

Probes specific for mouse *Saa1*, *Saa2*, *Saa3*, *Saa4*, and the 5′ and 3′ UTRs of *Saa5* were generated by PCR (10 mM Tris-HCl, pH 8.3, 2 mM $MgCl_2$, 50 mM KCl, 200 mM each dNTP, 0.5 μM each forward and reverse primers, and 4 μg/ml A/J genomic DNA), using the locus-specific primer pairs described previously. PCR conditions were 94 °C for 7 min, followed by 40 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1.2 min. Probes were then electrophoresed in 1% SeaPlaque agarose (Flowgen Instruments, Staffordshire, England), gel excised, and purified using the Wizard PCR Preps DNA purification system (Promega, Madison, WI). An oligolabelling kit (Pharmacia Biotech, Piscataway, NJ) was used to label 50 ng of each probe to high specific activity using [α^{32}P]dCTP (Amersham), before purification using Biospin 6 columns (BioRad Laboratories, Richmond, CA).

Sequence analyses

Mouse *Saa* sequences were obtained from the literature and the GenBank/EMBL DNA sequence database, accessed through the ACNUC retrieval system (Gouy et al. 1985), and subjected to pairwise alignment using the program CLUSTALW (Thompson et al. 1994).

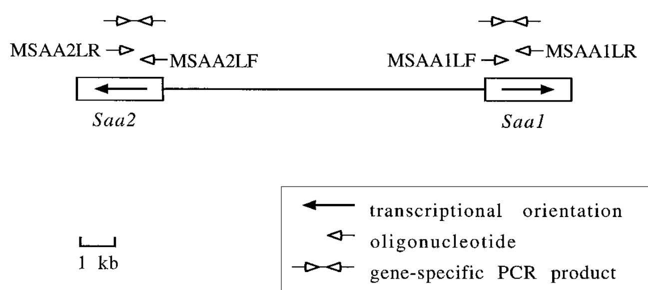

Fig. 1 Example of the long-range PCR mapping strategy employed. This represents the particular procedure used to verify the 9.5 kb mouse *Saa2/Saa1* linkage group previously described by Yamamoto and co-workers (1986)

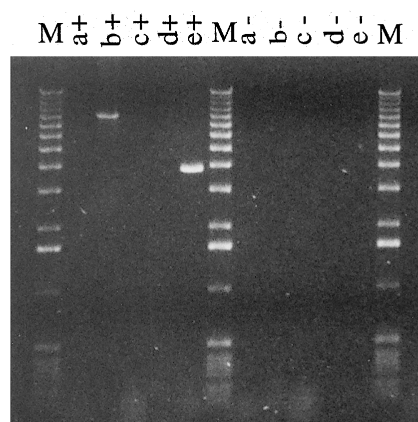

Fig. 2 Results of ethidium bromide stained agarose gel electrophoresis of long-range PCR products generated by *Saa1-Saa2* interlocus PCR reactions with A/J DNA as template in positive reactions (+), and sterile water as template in negative control reactions (–). The following PCR oligonucleotide pairs were used in the assay; *a* MSAA1LF and MSAA2LF; *b* MSAA1LR and MSAA2LR; *c* MSAA1LF and MSAA2LR; *d* MSAA1LR and MSAA2LF; *e* MSAA5*Eco*RIF and MSAA5*Eco*RIR (positive control reaction). *M* represents the 1 kb DNA ladder used as a size marker

The sequence accession numbers for mouse *Saa4* (Lowell et al. 1986b), mouse *Saa5* (de Beer et al. 1994), and human *SAA4* (Steel et al. 1993) are M13524, U02554, and L05920, respectively.

Results

Mouse *Saa* genes were mapped by locus-specific Expand Long Template PCR on A/J DNA, a technique enabling amplification of DNA fragments of up to 27 kb (Barnes 1994; Cheng et al. 1995). The PCR strategy, as applied to a previously described *Saa2/Saa1* linkage (Yamamoto et al. 1986), is illustrated in Figure 1. Briefly, locus-specific sense and anti-sense oligonucleotides were designed for each gene and *Saa1-Saa2* interlocus PCRs were performed using all possible pairwise combinations of these primers (i. e., MSAA1LF/MSAA2LF, MSAA1LR/MSAA2LR, MSAA1LF/MSAA2LR, and MSAA1LR/MSAA2LF). Furthermore, the products of internal (intralocus) PCR reactions (i. e., MSAA1LF/MSAA1LR and MSAA2LF/MSAA2LR) were used as *Saa* locus-specific probes in hybridizations to confirm the identity of any detected interlocus PCR products.

This strategy defines not only the map distance between assayed loci, but also their relative transcriptional orientations. For example, given the reported divergent transcriptional orientations of *Saa1* and *Saa2*, only the PCR assay utilizing the primers MSAA1LR and MSAA2LR should yield a product, since it is the only reaction in which the primers are juxtaposed when annealed to genomic DNA. Results of the *Saa1-Saa2* interlocus PCR confirmed the divergent transcriptional orientation of these genes (Fig. 2). The MSAA1LR/MSAA2LR PCR product was sized at approximately 8.5 kb which, when account is taken of the intragenic positions of the oligonucleotides used, corresponds to a distance of 9.3 to 9.4 kb separating the transcriptional start sites of the mouse *Saa1* and *Saa2* genes, in approximate agreement with Yamamoto and co-workers (1986).

Following confirmation of the 3′-*Saa2*-5′/5′-*Saa1*-3′ linkage group, these loci were assayed, by PCR, for their relative linkages to *Saa3*, *Saa4*, and *Saa5*, arbitrarily using the *Saa1* gene as a reference point. Only the MSAA1LF/MSAA4LR reaction yielded detectable PCR product, which was sized at roughly 9.8 kb, indicating an *Saa1-Saa4* intragenic distance of approximately 5.1 kb and establishing a 3′-*Saa2*-5′/5′-*Saa1*-3′/5′-*Saa4*-3′ linkage group (Fig. 3 A). Note that since *Saa4* is a pseudogene, the designation of 5′ to 3′ orientation is based, not upon transcriptional orientation per se, but on the results of sequence alignments with the mouse *Saa1*, *Saa2*, and *Saa3* genes (Lowell et al. 1986b).

PCR assays were next conducted between the loci *Saa2/Saa3*, *Saa2/Saa5*, *Saa4/Saa3*, and *Saa4/Saa5*, since *Saa2* and *Saa4* were the most distantly placed genes in the thus far established linkage group. This analysis yielded a MSAA4LF/MSAA5LR5′B PCR product of close to 2.3 kb, establishing that mouse *Saa4* and *Saa5* lie head-to-tail with respect to transcriptional orientations, and in exceptionally close proximity (Fig. 3 A). Having defined a 3′-*Saa2*-5′/5′-*Saa1*-3′/5′-*Saa4*-3′/5′-*Saa5*-3′ linkage group, PCR assay between *Saa3* and the 3′ UTR of *Saa5* yielded an MSAA5LF/MSAA3LR product of approximately 15.0 kb, and thus successfully integrated the *Saa3* locus into the mouse *Saa* map (Fig. 3 A). Finally, since only partial cDNA sequence data has been published to date for mouse *Saa5* (de Beer et al. 1994), Expand Long Template PCR was used to establish a distance of 3.85 kb between the MSAA5*Eco*-RIF and MSAA5*Eco*RIR oligonucleotides. The distance between the point of transcription initiation and the polyadenylation signal of mouse *Saa5* was thus determined to be approximately 4.7 kb, of which 2.2 kb encodes *Saa5* messenger RNA (mRNA), as defined by the reported size of *Saa5* mRNA (de Beer et al. 1994); the remaining 2.5 kb is therefore intronic (Fig. 3 A).

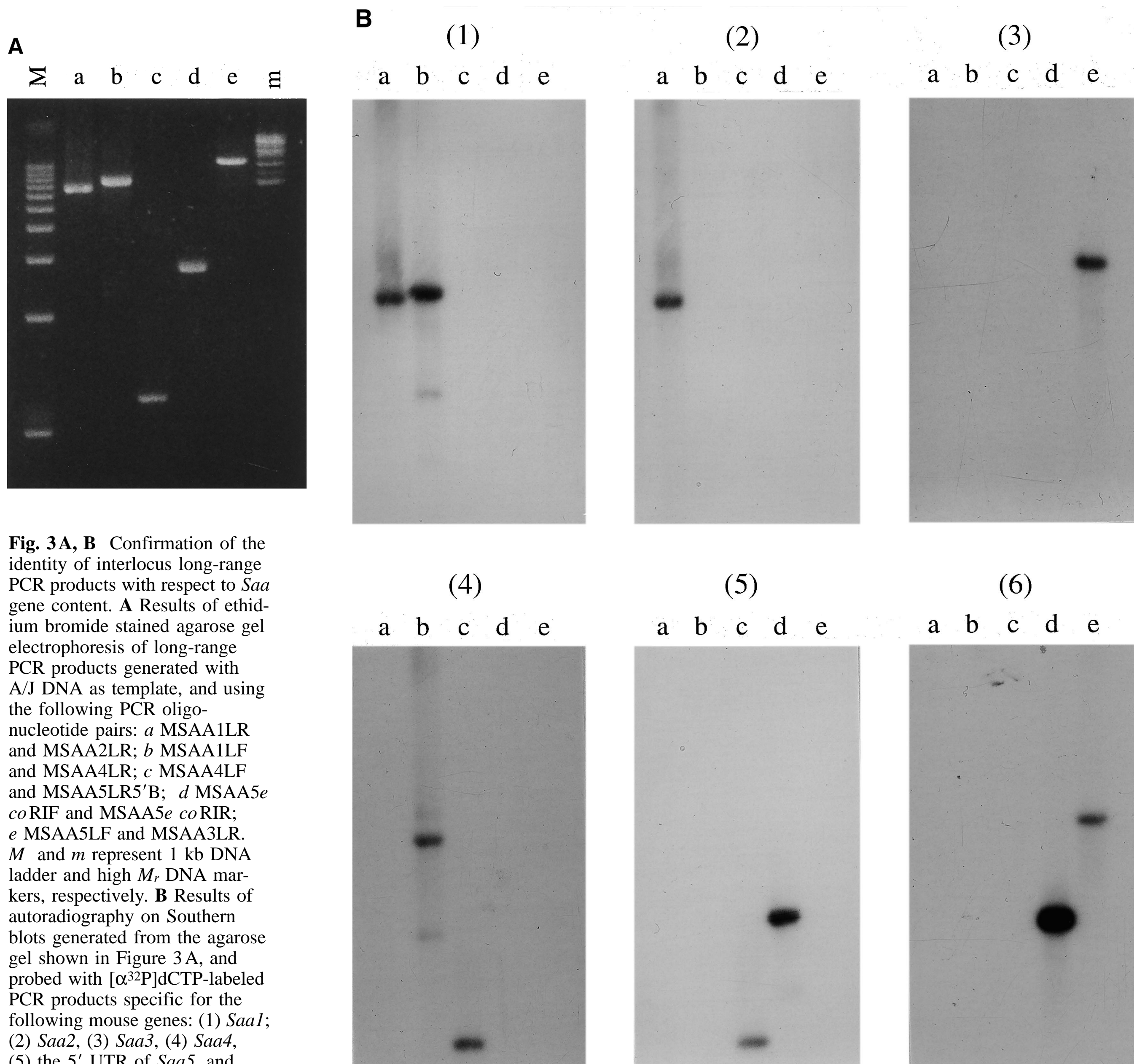

Fig. 3 A, B Confirmation of the identity of interlocus long-range PCR products with respect to *Saa* gene content. **A** Results of ethidium bromide stained agarose gel electrophoresis of long-range PCR products generated with A/J DNA as template, and using the following PCR oligonucleotide pairs: *a* MSAA1LR and MSAA2LR; *b* MSAA1LF and MSAA4LR; *c* MSAA4LF and MSAA5LR5′B; *d* MSAA5*eco*RIF and MSAA5*eco*RIR; *e* MSAA5LF and MSAA3LR. *M* and *m* represent 1 kb DNA ladder and high M_r DNA markers, respectively. **B** Results of autoradiography on Southern blots generated from the agarose gel shown in Figure 3 A, and probed with [α^{32}P]dCTP-labeled PCR products specific for the following mouse genes: (1) *Saa1*; (2) *Saa2*, (3) *Saa3*, (4) *Saa4*, (5) the 5′ UTR of *Saa5*, and (6) the 3′ UTR of *Saa5*

Confirmation of the identity of all PCR products with respect to *Saa* gene content was accomplished by Southern blotting and gene-specific hybridization. As expected, the *Saa1*-specific probe detected the MSAA1LR/MSAA2LR and MSAA1LF/MSAA4LR PCR products; the *Saa2* and *Saa3* PCR probes hybridized, respectively, to the MSAA1LR/MSAA2LR and MSAA5LF/MSAA3LR bands; the *Saa4* probe detected both MSAA1LF/MSAA4LR and MSAA4LF/MSAA5LR5′B PCR products; the 5′-*Saa5* probe hybridized to the MSAA4LF/MSAA5LR5′B and MSAA5*Eco*RIF/MSAA5*Eco*RIR products; and the 3′-*Saa5*-specific PCR probe annealed to both the MSAA5*Eco*RIF/MSAA5*Eco*RIR and MSAA5LF/MSAA3LR PCR products (Fig. 3 B).

On the basis of the above results, a linkage map of the mouse *Saa* gene cluster was constructed, in which the transcriptional orientations of all loci (or, in the case of *Saa4*, the ancestral gene transcriptional orientation presumed from sequence alignment with *Saa1*, *Saa2*, and *Saa3*) were defined (Fig. 4). It was thus established that the five mouse *Saa* genes map within a maximum 45 kb of each other, in the order 3′-*Saa2*-5′/5′-*Saa1*-3′/5′-*Saa4*-3′/5′-*Saa5*-3′/5′-*Saa3*-3′.

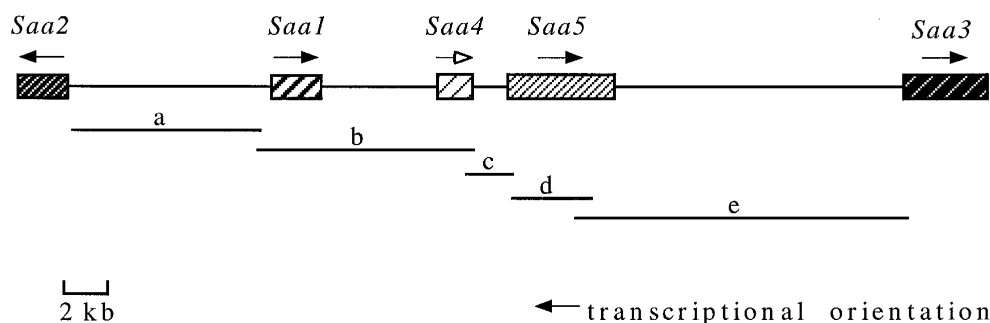

Fig. 4 Map of the mouse *Saa* gene cluster, generated by long-range PCR. Boundaries of genes are defined by their transcription initiation and polyadenylation signals, except in the case of the pseudogene *Saa4*, the boundaries of which are defined by the extremes of the *Saa4* sequence published by Lowell and co-workers (1986b). The positions of overlapping PCR products which were generated in the construction of the map are also indicated as follows: *a* MSAA1LR/MSAA2LR PCR product; *b* MSAA1LF/MSAA4LR PCR product; *c* MSAA4LF/MSAA5LR5′B PCR product; *d* MSAA5*Eco* RIF/MSAA5*Eco* RIR PCR product; *e* MSAA5LF/MSAA3LR PCR product

Discussion

In the present report, a recently described technique for the amplification of large DNA fragments was used to characterize the mouse *Saa* cluster, which was found to contain the genes 3′-*Saa2*-5′/5′-*Saa1*-3′/5′-*Saa4*-3′/5′-*Saa5*-3′/5′-*Saa3*-3′, in that order. The Expand PCR system uses an enzyme mixture of thermostable DNA polymerases, and is capable of amplifying fragments of up to 27 kb from genomic DNA (Barnes 1994; Cheng et al. 1995). To our knowledge, this is the first study which has applied this system to the dissection of gene order within a linkage group.

There are several advantages in adopting such an approach, since the concurrent determination of both the distance between, and the relative transcriptional orientations of, adjacent loci is facilitated. In addition, distances between genes may be more accurately defined than is possible with alternative techniques (such as long-range restriction digestion mapping), since precise positions of oligonucleotides used in the PCR assay are known, and PCR products may be sized accurately using agarose electrophoresis with appropriate size markers. The Expand PCR system is currently limited, however, to the assessment of gene clusters in which adjacent loci lie no more than 27 kb apart, although amplification of fragments larger then 27 kb may be possible in the future (Cheng et al. 1995). Thus, although the present study successfully produced a map of the mouse *Saa* region using this technique, it would not be applicable to characterization of, for example, the human *SAA* gene cluster (Sellar et al. 1994). It is clear, therefore, that this approach is useful only in the dissection of tightly clustered linkage groups, although broader applications will be possible if technical improvements are made in the future.

We have established that the genes encoding the mouse SAAs lie within 45 kb of each other on Chr 7 in the order 3′-*Saa2*-5′/5′-*Saa1*-3′/5′-*Saa4*-3′/5′-*Saa5*-3′/5′-*Saa3*-3′. This tight linkage is likely to be of considerable functional importance, since Lowell and co-workers (1986a) have described co-ordinate transcriptional regulation of the acute-phase *Saa* genes during the APR. *Saa5* expression in the liver is also known to exhibit a rapid, albeit muted, response to inflammatory stimuli (de Beer et al. 1994). These observations, taken together with the fact that enhancer sequences of higher eukaryotic genes can increase transcription of genes located at least 69 kb upstream or downstream (Serfling et al. 1985; Winoto and Baltimore 1989), suggest that the mouse *Saa* genes may have a common transcriptional regulator(s). Precedent exists for the clustering of eukaryotic genes with a common transcriptional regulator in close proximity of each other. For example, major histocompatibility genes are transcriptionally regulated by interferon (IFN) and map, within a conserved linkage group, to human Chr 6 and mouse Chr 17 (Peska et al. 1987). Furthermore, the observation that the SAA-encoding genes of both mouse and human exist in tight clusters suggests that selective pressure may maintain genes which are under coordinate transcriptional control within close physical proximity.

Comparison of the mouse *Saa* gene cluster map with that of human *SAA*s (Sellar et al. 1994) reveals that the former is considerably more compact, spanning no more than 45 kb, in contrast to at least 150 kb for the human gene cluster. Examination of relative map positions alone suggest that the evolutionary handogues of the mouse *Saa1* and *Saa2* genes are the human *SAA2* and *SAA1* genes, respectively. Furthermore, the maintenance of the same relative transcriptional orientation in both species suggests that there have been no gross rearrangements, such as duplications or inversions, affecting these loci since divergence of the common ancestor to give the human and mouse lineages. Previous evolutionary analyses, by computer-based comparison of nucleotide and protein sequences, failed to identify the human and mouse acute phase gene homologues, since their sequences are highly similar (Uhlar et al. 1994). Thus, comparison of the positional information for mouse and human SAA-encoding genes provides the best means, at present, of suggesting homologues for mouse *Saa1* and *Saa2*.

The human *SAA3* gene, although occupying a position within the human cluster comparable with that of the mouse *Saa3* location presented here, is a pseudogene, and therefore is clearly not a modern functional homologue of mouse *Saa3* (Sack and Talbot 1989; Kluve-Beckerman et al. 1991;

Sellar and Whitehead 1993). However, human *SAA3* is a pseudogene by virtue of a minor mutation, namely a single base insertion. Furthermore, *SAA3* sequences defined in three non-human primates indicate that the insertion is present in two higher primates, the chimpanzee and gorilla, while it is absent in an Old World primate, the macaque (Kluve-Beckerman et al. 1991). This suggests that human *SAA3* is a pseudogene by virtue of a mutation that is very recent in evolutionary terms. Thus, the mouse *Saa* and human *SAA3* genes may have been functional homologues in the comparatively near past, although the *SAA3* gene product is clearly no longer essential in human.

Comparative map positions, together with relative transcriptional orientations, suggest that either mouse *Saa4* or *Saa5* may be the evolutionary homdogue of the human constitutively expressed *SAA4* gene (Whitehead et al. 1992; Steel et al. 1993). Therefore, pairwise alignments were performed between exon 3 and exon 4 sequences of these genes. Optimal alignment of human *SAA4* and mouse *Saa4* necessitated the introduction of a 24 base pair (bp) gap in the pseudogene sequence, corresponding to the position of a previously characterized octapeptide insert in the SAA4 protein (Steel et al. 1993). On this evidence alone we consider it unlikely that the mouse pseudogene represents an evolutionary homdogue of human *SAA4*. In contrast, sequence alignment of mouse *Saa5* and human *SAA4* indicates that these genes may have been functional homologues, since no gap needs to be introduced for optimal alignment, and 73% nucleotide identity is evident across the exon 3 and 4 sequences. Caution must be exercised, however, since the degree of protein sequence divergence between mouse Saa5 and human SAA4 is striking when compared with the limited divergence between, for example, the mouse and human genes which encode A-SAAs (Uhlar et al. 1994).

A final noteworthy feature of the mouse *Saa* gene cluster map presented here is the extremely short interval which separates the mouse *Saa4* pseudogene and the 5′ end of mouse *Saa5* (approximately 1.7 kb). This suggests that elements which regulate the transcription of *Saa5* may reside within the published pseudogene sequence (Lowell et al. 1986b), as well as in the sequence separating these genes. Since SAA5 is essentially constitutively expressed in liver, is present as a normal component of HDL_3, and exhibits very different acute phase induction kinetics to those of the mouse A-SAA proteins, it is of considerable interest to characterize any transcriptional elements which may contribute to the control of expression of mouse SAA5. Thus, we are currently extending our characterization of the mouse *Saa* gene cluster by sequencing the region between *Saa4* and the 5′ end of *Saa5*. Sequence derived from this analysis, as well as that published for *Saa4*, will then be subjected to computer-based analyses, in the hope of identifying transcriptional control elements for mouse *Saa5*.

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