FEBS Letters 446 (1999) 142–148 FEBS 21665

Murine 12(*R*)-lipoxygenase: functional expression, genomic structure and chromosomal localization

Peter Krieg*, Malte Siebert, Andreas Kinzig, Rainer Bettenhausen, Friedrich Marks, Gerhard Fürstenberger

Research Program on Tumor Cell Regulation, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany

Received 14 December 1998; received in revised form 3 February 1999

Abstract A cDNA, recently cloned (by Krieg et al. (1998)) from mouse skin, was shown to encode a 12(*R*)-lipoxygenase. When expressed in HEK cells, the recombinant protein converted methyl arachidonate into the corresponding 12-HETE ester which was shown to be the *R*-enantiomer by chiral phase chromatography. Neither arachidonic acid nor linoleic acid were substrates for the recombinant protein. The structure of the 12(*R*)-lipoxygenase gene is unique among all animal lipoxygenases in that it is divided into 15 exons and 14 introns spanning approximately 12.5 kb. By interspecific backcross analysis, the 12(*R*)-lipoxygenase gene was localized to the central region of mouse chromosome 11.

Key words: Lipoxygenase; Functional expression; Gene structure; Chromosomal localization

1. Introduction

Lipoxygenases represent a widely distributed family of non-heme, non-sulfur, iron containing dioxygenases that catalyze the regio-selective and stereo-selective dioxygenation of fatty acid substrates containing one or more (Z,Z)-1,4-pentadiene moieties. The primary reaction products are hydroperoxides of conjugated (E,Z)-dienes that are further converted to a variety of metabolites. Many lipoxygenase metabolites are potent endogenous signalling molecules involved in the control of vasoconstriction, bronchoconstriction, blood clotting, as well as granulocyte chemotaxis and other inflammatory reactions [1]. Irregular expression of lipoxygenases and the presence of their products are also associated with a series of diseases including atherosclerosis [2,3], inflammatory bowel disease [4], asthma [5], psoriasis [6,7] and cancer [8,9].

Mammalian lipoxygenases are named according to the positional specificity of oxygen insertion into arachidonic acid. There are four well characterized lipoxygenase isoenzymes preferentially expressed in blood cells, i.e. 5-lipoxygenase [10], 15-lipoxygenase [11], the closely related leukocyte type 12-lipoxygenase and platelet type 12-lipoxygenase [12]. Mammalian skin turned out to be another tissue with extensive lipoxygenase-catalyzed fatty acid metabolism. Four additional lipoxygenase isoenzymes have been characterized and cloned so far from this source, including a 15-lipoxygenase-2 from human skin [13], an epidermis type 12-lipoxygenase [14,15], a phorbol ester inducible 8-lipoxygenase [16,17] and a hitherto enzymatically unspecified epidermis type lipoxygenase-2 (e-LOX-2) from mouse skin [17]. With 701 amino acid residues the latter enzyme is unique among all mammalian lipoxygenases. The larger size has been attributed to an additional proline-rich stretch of 31 amino acids inserted between residues P^{154} and P^{185} which does not correspond to any other known plant or mammalian lipoxygenase sequences.

Here we show that e-LOX-2 encodes a 12(*R*)-lipoxygenase which represents the mouse ortholog of the recently published human 12(*R*)-lipoxygenase [18] and which, in contrast to conventional lipoxygenases, prefers arachidonyl ester instead of arachidonate as substrate. Analysis of the mouse 12(*R*)-lipoxygenase gene (*Alox12b*) revealed unique structural features indicating that *R*-lipoxygenases may represent a distinct subfamily of the mammalian lipoxygenase multigene family.

2. Materials and methods

2.1. Expression of lipoxygenase cDNA

For transient expression, plasmid DNA (10 μg) and vector DNA, as control, were introduced into human embryonic kidney (HEK) 293 cells (seeded at 1.5×10^6 cells/100 mm plate) by a modified calcium phosphate transfection procedure using a mammalian transfection kit according to the manufacturer's protocol (Stratagene). Cells were harvested 48 h after transfection and homogenized by sonication (Branson Sonifier, 10 pulses for 6 s on ice) in TE buffer (50 mM Tris-HCl, 1 mM EDTA, 1 mM EGTA, 10 μg/ml leupeptin, pH 7.4).

2.2. Western blot analysis

Protein aliquots (80 μg) of cell homogenates from transfected cells were electrophoresed in 7.5% SDS-polyacrylamide gels. The proteins were electroblotted onto PVDF membranes and these were subsequently treated as per the protocol, recommended by the supplier of the ECL detection system (Amersham Life Science). The membranes were probed using a rabbit polyclonal antibody raised against the human 12-lipoxygenase (Alexis). This antibody recognizes murine platelet type and leukocyte type 12(*S*)-lipoxygenases [15] as well as the murine 12(*R*)-lipoxygenase. The anti-12-lipoxygenase antiserum was used at a dilution of 1:6000 and the peroxidase-conjugated goat anti-rabbit IgG (H+L) antibody (Jackson Laboratories) at a dilution of 1:2000.

2.3. Lipoxygenase assay

Aliquots of 250 μl of cell homogenates were incubated in the presence of arachidonic acid (100 μM, 20 μM or 4 μM), linoleic acid (100 μM) or arachidonic acid methyl ester (100 μM) for 12 min at 37°C. The incubations were terminated by the addition of 40 μl of 1 M sodium formiate buffer (pH 3.1). To reduce the hydroperoxy acids to hydroxy acids, 20 μl of trimethylphosphate was added and incubated at room temperature for 5 min. Samples were extracted twice by ethylacetate. The combined organic extracts were evaporated and an-

*Corresponding author. Fax: (49) (6221) 42 4406.
E-mail: p.krieg@dkfz-heidelberg.de

The approved gene symbol for mouse 12(*R*)-lipoxygenase by MGD is *Alox12b*. The nucleotide sequence reported in this paper has been submitted to the GenBank/EMBL Data Bank with accession number Y18477. Mapping data from this article have been deposited in the Mouse Genome Database under accession number J:46510.

0014-5793/99/$20.00
PII: S0014-5793(99)00196-9

alyzed by reverse phase HPLC, straight phase HPLC and chiral phase chromatography.

2.4. Analysis of HETEs and HETE methyl esters

Reverse phase HPLC analysis of the hydroxy-eicosatetraenoic acid (HETE) fraction was done on a 5 μm YMC-Pack ODS-AM column with a 1 cm guard column (25×0.46 cm, YMC Europe, Schermbeck, Germany) using the solvent system of methanol/water/glacial acetic acid (85/15/0.01 by volume) and a flow rate of 0.5 ml/min. The 12-HETE fraction was re-chromatographed by straight phase HPLC using a Zorbax Sil column (25×0.46 cm, 5 μm, Bischoff, Stuttgart, Germany) eluted with 1 ml/min of the solvent system n-hexane/isopropanol/acetic acid/H_2O (98/2/0.1/0.025 by volume) and then analyzed by chiral phase chromatography using a Chiralcel OB column (25×0.46 cm, 5 μm, J.T. Baker, Deventer, The Netherlands) with the solvent system n-hexane/isopropanol/acetic acid (99/1/0.05 by volume, flow rate 0.5 ml/min). Elution of the HETEs was monitored at 236 nm.

2.5. Polymerase chain reaction (PCR) amplification and sequencing

To characterize the intron/exon organization of the *Alox12b* gene, mouse genomic DNA was subjected to PCR amplification using primers derived from within adjacent exons. In addition, several overlapping fragments were generated using primers designed from the mouse full length cDNA coding sequence.

Standard PCR was primed with 10 ng template DNA using 10 pmol of primers, 10 mM Tris, pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 0.2 mM gelatin, with 0.2 mM of each dNTP and 5 U *Taq* DNA polymerase (Appligene-Oncor) in a 50 μl reaction. Typical cycling profiles were programmed in a PTC-200 DNA Engine (MJ Research) as follows: 94°C for 5 min (1 cycle), 94°C for 1 min, 55–58°C for 1 min, 72°C for 1 min (30–35 cycles), 72°C for 10 min (1 cycle). Longer PCR products (introns 2, 7 and 11) were obtained using the Expand High Fidelity PCR system (Boehringer Mannheim, Mannheim, Germany) following the manufacturer's recommendations. The resulting PCR products were analyzed on agarose gel and the appropriate bands were excized, purified (Qiaquick gel extraction kit, Qiagen) and cloned into the PCRII TOPO or PCR XL-TOPO vector (Invitrogen) prior to sequencing.

Sequences were determined on both strands using the ABI Big Dye Terminator Cycle Sequencing Ready Reaction kit and the products were resolved on an ABI prism 310 Genetic analyzer (Perkin-Elmer/Applied Biosystems). The sequences were assembled and analyzed using the Heidelberg Unix Sequence Analysis Resources software programs.

2.6. Genomic library screening

Approximately 10^6 plaques of a mouse NMRI genomic library, in the lambda vector EMBL3, were screened by filter hybridization with purified insert of the full length cDNA clone of mouse 12(*R*)-lipoxygenase and two clones were purified by re-screening. PCR amplification of bands corresponding to 5′ and 3′ sequences of the cDNA indicated that clone λL2 contained most of the encoding sequences of the 12(*R*)-lipoxygenase including the amino-terminal domains but lacked the carboxy-terminal sequence. A 4.5 kb *Eco*RV/*Eco*RI fragment harboring 3.5 kb of the 5′ flanking region of the gene was subcloned into pBluescript and sequenced.

2.7. Chromosomal localization

DNA from C57BL/6J and *Mus spretus* mice (obtained from The Jackson Laboratory) was digested with several enzymes and analyzed for informative restriction fragment polymorphism by Southern blot analysis essentially as described [19]. The probe for *Alox12b* was the gel electroeluted and purified insert of the cDNA clone of mouse 12(*R*)-lipoxygenase [17] labelled with α-[^{32}P]dCTP using the multiprime DNA labelling system (Amersham). Washing was performed to a final stringency of 0.5×SSC, 0.5% SDS at 60°C. *Hin*dIII digestion of C57BL/6J DNA produced fragments of 10 kb and 4 kb: *M. spretus* DNA yielded fragments of 10 kb, 5 kb and 1.6 kb. The final interspecific backcross mapping was performed on the BSS interspecific backcross panel ((C57BL/6JEi×SPRET/Ei)F1 female x SPRET/Ei male) obtained from The Jackson Laboratory [20] and the presence or absence of the C57BL/6J-specific 4 kb fragment was followed in backcross mice. The data were submitted to The Jackson Laboratory and linkage data were obtained for *Alox12b*.

3. Results

3.1. Functional expression of murine 12(R)-lipoxygenase

From phorbol ester-treated mouse epidermis, we have recently cloned a cDNA encoding a 701 amino acid lipoxygenase isoenzyme tentatively called e-LOX-2 [17]. The eukaryotic expression vector pcDNA3 carrying the cDNA under the control of the cytomegalovirus promoter was used to transiently express e-LOX-2 cDNA in HEK 293 cells. Transfected cells expressed large amounts of recombinant protein as shown by Western blot analysis using a cross reacting anti-12-lipoxygenase antiserum (Fig. 1). Upon incubation of sonic extracts from transfected cells expressing 12(*R*)-lipoxygenase (Fig. 1, lane 2) with varying concentrations of arachidonic acid, no lipoxygenase-catalyzed products could be detected (Fig. 2). 15-HETE generated in lysates from both 12(*R*)-lipoxygenase expressing and control cells was found to be racemic, as previously reported [15]. However, when methyl arachidonate was used as substrate, 12-HETE methyl ester and its hydrolysis product 12-HETE were generated (Fig. 3). In addition, minor amounts of 8-HETE methyl ester and 8-HETE were detected. Formation of 8- and 12-HETE was due to a lipase activity present in the HEK 293 cell lysate. The oxygenated products were identified by co-migration of authentic standards on reverse phase HPLC (Fig. 3) and straight phase HPLC (not shown). 12-HETE methyl ester (Fig. 3D) and 12-HETE (Fig. 3C) were almost exclusively the *R* enantiomer, thereby identifying e-LOX-2 as a 12(*R*)-lipoxygenase.

3.2. Genomic structure

All known animal lipoxygenase genes are divided into 14 exons and 13 introns in the same positions [21]. The genomic organization of the mouse 12(*R*)-lipoxygenase was determined by PCR amplification of genomic fragments with cDNA-derived primers corresponding to sequences flanking the highly conserved intron positions. In addition, overlapping frag-

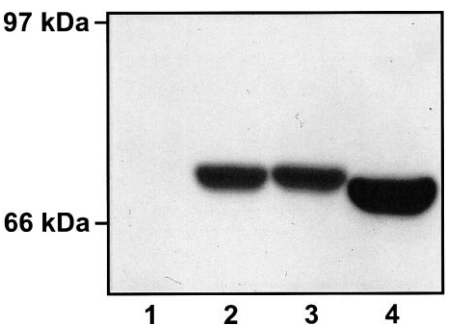

Fig. 1. Expression of recombinant murine 12(*R*)-lipoxygenase and platelet type 12-lipoxygenase in HEK 293 cells. HEK 293 cells were transfected with pcDNA3 vector and with pcDNA3 constructs carrying the murine 12(*R*)-lipoxygenase or the platelet type 12-lipoxygenase cDNAs. Aliquots of cell sonicates (80 μg protein) incubated with arachidonic acid or with methyl arachidonate were electrophorized in 7.5% SDS-polyacrylamide gels. The proteins were electroblotted onto a PVDF membrane and incubated with an anti-human platelet type 12-lipoxygenase antiserum. Protein was detected by an enhanced chemiluminescence method (see Section 2). Sonicates of cells transfected with vector pcDNA3 and incubated with arachidonic acid, lane 1; transfected with 12(*R*)-lipoxygenase and incubated with arachidonic acid, lane 2; transfected with 12(*R*)-lipoxygenase and incubated with methyl arachidonate, lane 3; transfected with platelet type 12-lipoxygenase and incubated with arachidonic acid, lane 4.

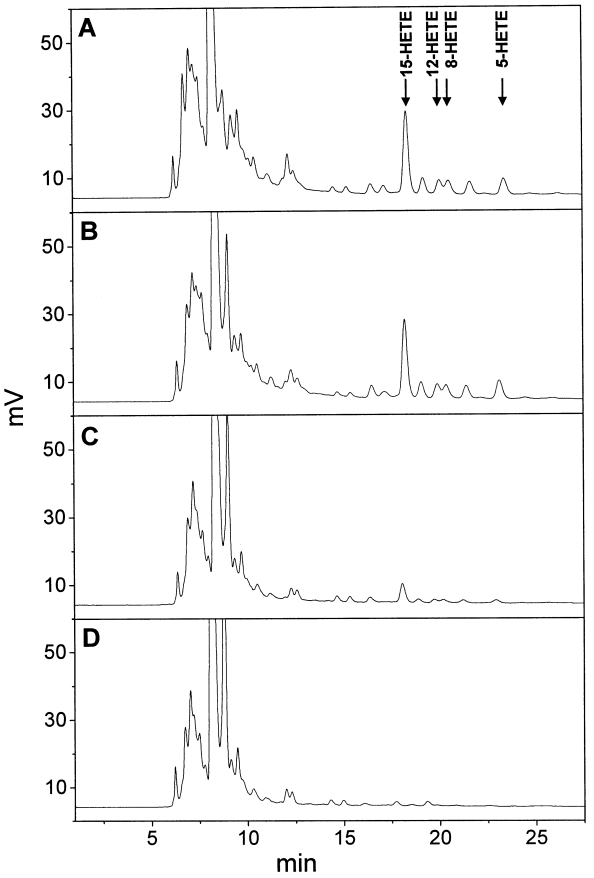

Fig. 2. Characterization of products formed from arachidonic acid by HEK 293 cells expressing murine 12(*R*)-lipoxygenase. Homogenates from HEK 293 cells transiently transfected with vector constructs without insert (A) or with constructs containing 12(*R*)-lipoxygenase cDNA (B–D) were incubated in TE buffer with 100 μM arachidonic acid (A, B), 20 μM arachidonic acid (C) or 4 μM arachidonic acid (D) for 12 min at 37°C. Upon reduction, lipids were extracted with ethyl acetate. The extracts were analyzed on a YMC-Pack reverse phase column as described in Section 2. 5-, 8-, 12- and 15-HETE were used as internal standards. Racemic 15-HETE is generated both in homogenates from cells transfected with vector and with 12(*R*)-lipoxygenase cDNA.

ments spanning the complete coding region were generated to compare genomic and cDNA sequences revealing the presence of an additional intron inserted in exon 4. In accordance to the highly conserved exon/intron structure of the remaining gene, this additional intron was numbered 4a and the corresponding exonic sequences 4a and 4b (Fig. 4). Sequences around exon/intron junctions and the length of the introns were determined. The exon/intron boundaries all conform to the consensus splice donor/acceptor sequences and the total size of the gene is ~12.5 kb (Fig. 4, Table 1).

3.3. 5′ Flanking region of the murine 12(R)-lipoxygenase gene

By hybridization screening of a NMRI mouse genomic library with the cDNA probe for murine 12(*R*)-lipoxygenase we obtained several positive clones, one of which (λL2) contained most of the encoding sequence including the amino-terminal domain. A 4.5 kb *Eco*RV/*Eco*RI fragment of λL2 covering a 3.5 kb long 5′ flanking region (Fig. 5) was sequenced. An approximately 950 bp region extending upstream of the 12(*R*)-lipoxygenase encoding sequence to highly repetitive CT sequences found between positions −1308 and −953 is given in Fig. 5. The G+C rich putative promoter region lacked 100% consensus sequence match with TATA and CCAAT sequences and also with Sp-1 binding sites. Searches for *cis*-regulatory elements using TRANSFAC software pack-

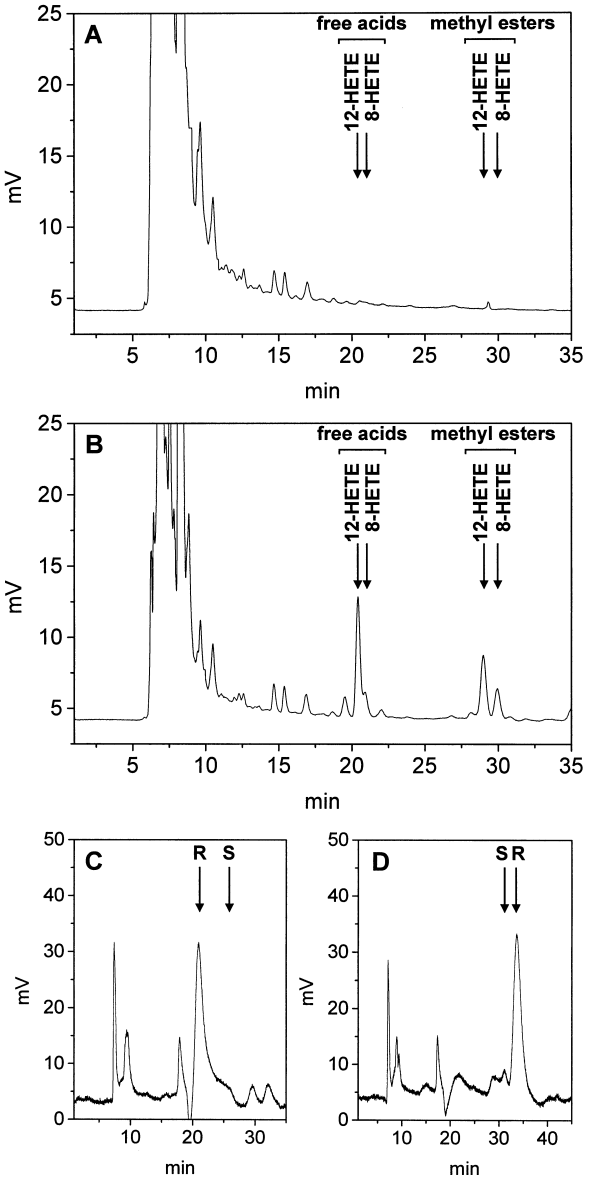

Fig. 3. Reverse and chiral phase HPLC chromatograms of the products formed by the murine 12(*R*)-lipoxygenase expressed in HEK 293 cells. Homogenates from HEK 293 cells transiently transfected with vector constructs without insert (A) or constructs containing 12(*R*)-lipoxygenase cDNA (B–D) were incubated in TE buffer with 100 μM arachidonic acid methyl ester for 12 min at 37°C. H(P)ETEs were reduced and extracted with ethyl acetate. The extracts were analyzed on a YMC-Pack reverse phase column as described in Section 2. 5-, 8-, 12- and 15-HETE as well as 8-, 12- and 15-HETE methyl ester were used as internal standards. The retention times of 12-HETE and 12-HETE methyl ester were 20.4 min and 29.0 min, respectively. The 12-HETE and 12-HETE methyl ester fractions were subjected to chiral phase chromatography on a Chiracel OB column using 12(*S*)- and 12(*R*)-HETE (C) and the corresponding methyl esters (D) as internal standards. The retention times of 12(*R*)- and 12(*S*)-HETE were 20.6 min and 26.0 min and those of the 12(*S*)- and 12(*R*)- HETE methyl esters were 30.3 min and 33.8 min, repectively.

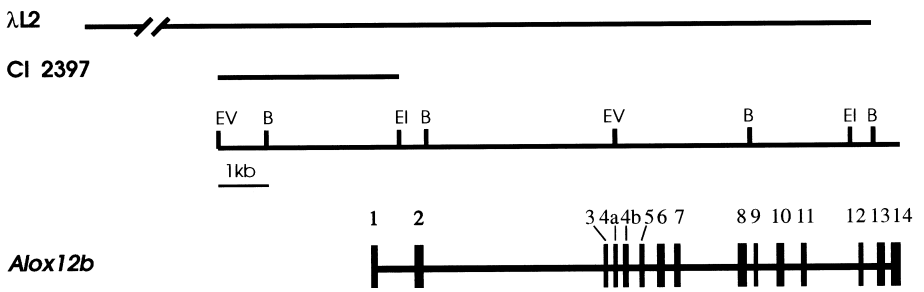

Fig. 4. Structure of the mouse 12(*R*)-lipoxygenase gene. The upper line designates the location of the genomic clone λL2 in relation to the sequence information. The *Eco*RV/*Eco*RI fragment that was subcloned into plasmid vector (Cl 2397) is shown underneath. The confirmed restriction enzyme sites are shown for *Bam*HI (B), *Eco*RI (EI) and *Eco*RV (EV). The third line illustrates the structure of the *Alox12b* gene. Introns are depicted by thick lines, exons by boxes. The exon and intron sizes are drawn to scale.

ages, revealed the presence of a number of potential binding sites for known transcription factors among them AP-2, GATA binding sites and silencer elements which have been identified in the promoter regions of 5-, 12- and 15-lipoxygenases [22–25].

3.4. Chromosomal localization of the murine 12(*R*)-lipoxygenase gene

The chromosomal localization of the mouse *Alox12b* gene was determined by typing the Jackson BSS interspecific backcross panel. DNA from C57BL/6J and *M. spretus* mice was digested with several enzymes and analyzed for informative restriction fragment polymorphism using the full length 12(*R*)-lipoxygenase cDNA as probe. A 4 kb *Hin*dIII C57BL/6J-specific RFLP was used to follow the segregation of the *Alox12b* locus in the backcross mice. The mapping results indicated that *Alox12b* is located in the central region of mouse chromosome 11 co-segregating with *C1qbp, Gucy2e, D11Mit4* and a number of EST loci (Fig. 6).

4. Discussion

Mammalian lipoxygenases are known to insert molecular oxygen regio specifically and enantio selectively into poly-unsaturated fatty acids with *S* chirality of the corresponding H(P)ETEs. On the other hand, lipoxygenases of invertebrate origin have been shown to generate either *S* or *R* enantiomeric reaction products. We and others have recently cloned cDNAs from mouse and human skin which encode proteins displaying sequence motifs being characteristic for lipoxygenases and exhibiting a 86% sequence identity [17,18]. An unique structural feature of both proteins is an extra domain

Table 1
Exon/intron organization of the mouse 12(*R*)-lipoxygenase gene

Exon/size (bp)					Intron/size (kb)				
1 (147)	ACG T	GGG G	GCG A^{49}	gtaagtgcgc...	I (0.882)	...ttttgtgcag	GTG V^{50}	GAT D	GAC D
2 (205)	GCC A	ACA T^{117}	G	gtgagccagc...	II (≈4.3)[a]	...ctcacgacag	GA G^{118}	AAG K	ATA I
3 (82)	TTC F	TAC Y	CA H^{145}	gtgagcggga...	III (0.143)	...ctctttgcag	C	TGG W^{146}	AGG R
4a (93)	CGG R	CCT P	GA E^{176}	gtaaggacca...	IVa (0.139)	...tttcccacag	G	TGG W^{177}	GAT D
4b (123)	GGG G	CCC P	AT M^{117}	gtgagtgcac...	IVb (0.265)	...atcctcccag	G	GCG A^{118}	CTG L
5 (104)	GTC V	TCA S^{251}	G	gtatggggtg...	V (0.316)	...cacttcctag	AG E^{252}	TAT Y	GTG V
6 (173)	GAG E	CTG L	GAG E^{309}	gtgaggtcct...	VI (0.231)	...tcccctccag	AGG R^{310}	GGG G	AAT N
7 (144)	GCC A	ATT I	CAG Q^{357}	gtaccaaggg...	VII (1.373)	...ccacctccag	CTC L^{358}	AGT S	CAG Q
8 (204)	CTG L	TAT Y	AAG K^{425}	gtaaaggttc...	VIII (0.175)	...ccctgcccag	CTT L^{426}	CTC L	ATC I
9 (87)	TCT S	GCC R	AGG R^{454}	gtaagactcc...	IX (0.457)	...ctccctcaag	GCC A^{455}	ATG M	TCC S
10 (170)	ATG M	GAG E	AG R^{511}	gtgaggtggg...	X (0.411)	...ttctgtgcag	G	TAC Y^{512}	GTG V
11 (122)	AGC S	TCA S^{551}	G	gtatgggtgt...	XI (1.250)	...aatccctcag	GC G^{552}	TTC F	CCC P
12 (101)	TCA S	GGC G	CAG Q^{585}	gtactcgcca...	XII (0.340)	...tccactctag	CTG L^{586}	GAG E	TAC Y
13 (171)	GAT D	GAC D	AGG R^{642}	gtacgggggt...	XIII (0.157)	...tgttctgcag	CGG R^{643}	CCG P	CTT L
14 (246)									

Exon/intron sizes and nucleotide sequences at the exon (upper case) and intron (lower case) boundaries are given. The size of exon 1 counted from the initiatiting ATG and the end of intron 14 to the first A of the poly(A) tail of the cDNA.
[a]This intron has not been completely sequenced.

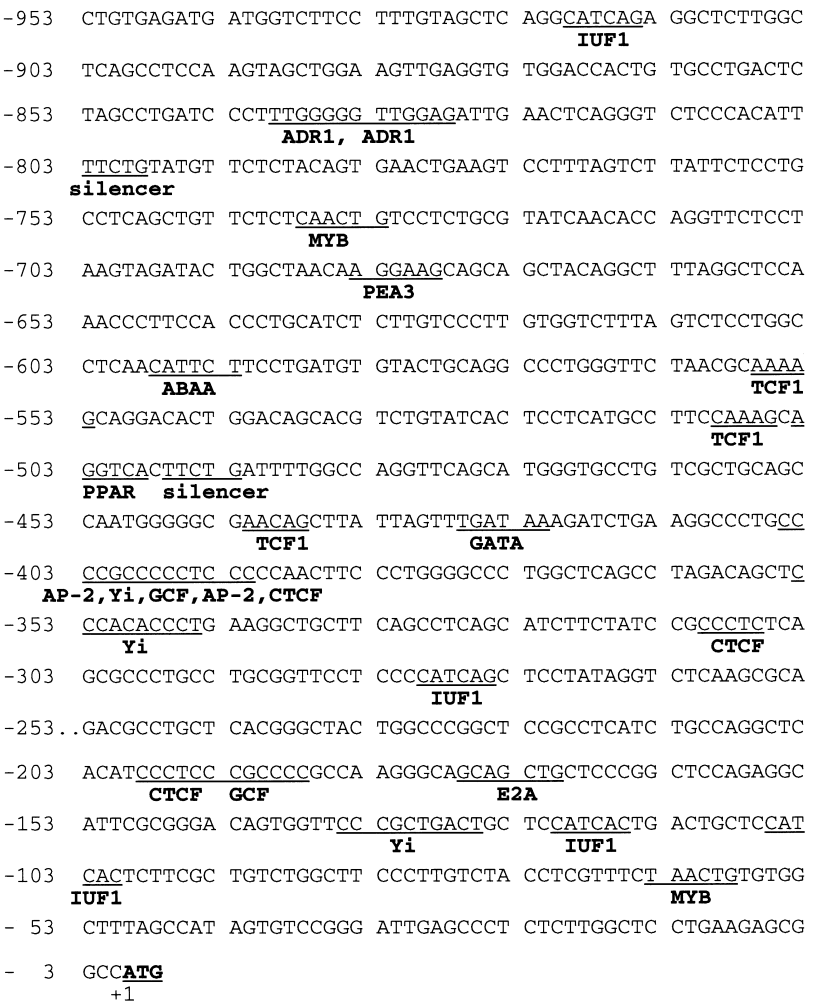

Fig. 5. 5′ Flanking region of the mouse 12(*R*)-lipoxygenase gene. A region spanning 953 bases from the end of a highly repetitive segment to the ATG initiator codon is displayed. The complete 3.5 kb fragment of the 5′ flanking sequence not shown in this figure is available at GenBank/EMBL Data Bank under the accession number Y18477. Nucleotides are numbered from the translational start codon. Consensus binding sequences for known transcription factors are underlined.

of 31 amino acids located in a loop region between helix 1 and 2 at the surface of the C-terminal catalytic domain near the proposed substrate binding cavity.

The human protein was found to metabolize arachidonic acid almost exclusively into 12(*R*)-HETE [18]. Here we show that the murine ortholog protein is inactive with arachidonic acid as substrate when expressed in HEK 293 cells.The same holds true for linoleic acid (data not shown). Enzymatic activity was also lacking when other expression systems were used including HeLa cells infected with vaccinia virus encoding the T7 RNA polymerase which provides an abundant source of the recombinant enzyme. However, when arachidonic acid methyl ester was used as a substrate, the corresponding 12(*R*)-HETE methyl ester and 12(*R*)-HETE were generated. Since free arachidonic acid was not accepted as a substrate, 12(*R*)-HETE most probably is the hydrolysis product of the corresponding methyl ester. In fact, both HEK 293 and HeLa cell lysates contained lipolytic activities hydrolyzing methyl arachidonate. Arachidonic acid esters are also accepted as substrates by the reticulocyte 15-lipoxygenase and leukocyte type 12-lipoxygenase [26–28]. Nevertheless, the latter enzymes preferentially oxygenate the free acid. The exclusive substrate specificity of mouse 12(*R*)-lipoxygenase may indicate that esterified rather than free fatty acids may represent the natural substrates of this enzyme. However, such arachidonyl esters do not include ordinary phospholipids since these have not been found to be oxygenated by this enzyme. 12(*R*)-lipoxygenase was found to represent the most abundant isoform among the lipoxygenases constitutively expressed in normal mouse skin [17]. In this context it is intriguing that the epidermal lipid barrier contains esterified polyunsaturated fatty acids and is impaired by inhibitors of lipoxygenases, an effect that can be restored by lipoxygenase products, e.g. 13(*S*)-HODE [29].

Whether or not the remarkable difference in substrate specificity between the human and mouse 12(*R*)-lipoxygenases may be attributed to distinct structural features of the proteins is presently unknown. It must be emphasized that, although the murine 12(*R*)-lipoxygenase appears to be selective for esterified arachidonic acid, the enzyme nevertheless exhibits only a low catalytic activity similar to other lipoxygenases of epidermal origin such as epidermis type 12(*S*)-lipoxygenase and 8-lipoxygenase [15–17]. One possibility is that the physiological substrates have not properly been identified.

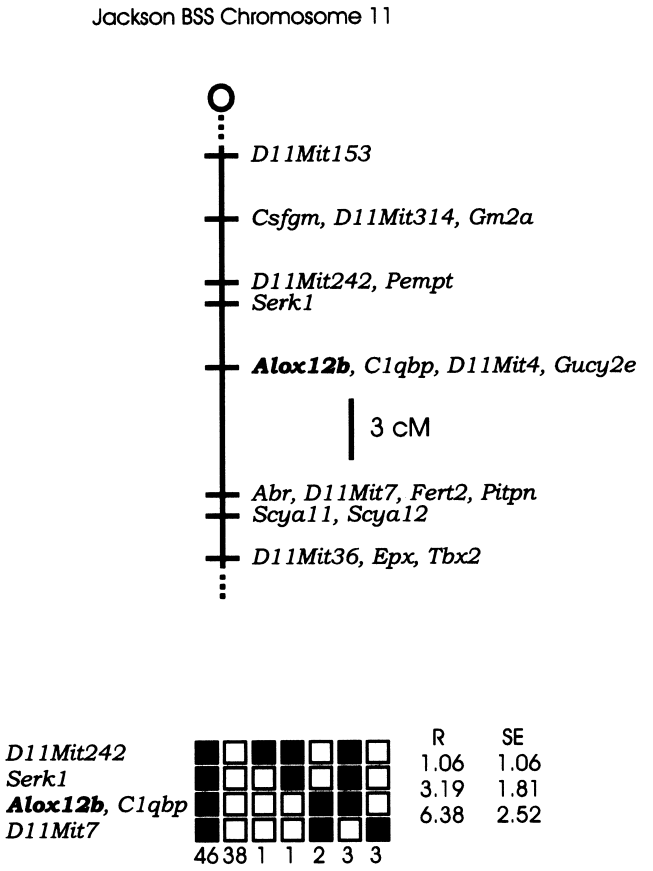

Fig. 6. Chromosomal localization of the mouse *Alox12b* gene. *Alox12b* maps to the central region of mouse chromosome 11 by interspecific backcross analysis. Top: map figure from The Jackson BSS backcross showing part of chromosome 11. The map is depicted with the centromere toward the top. A 3 cM scale bar is shown to the right of the figure. Loci mapping to the same position are listed in alphabetical order. Missing typings were inferred from surrounding data where assignment was unambiguous. Raw data from the Jackson Laboratory were obtained from the World Wide Web address http://www.jax.org/resources/documents/cmdata. Bottom: haplotype figure from the Jackson BSS backcross showing part of chromosome 11 with loci linked to *Alox12b*. Loci are listed in order with the most proximal at the top. The black boxes represent the C57BL6/JEi allele and the white boxes the SPRET/Ei allele. The number of animals with each haplotype is given at the bottom of each column of boxes. The percentage of recombination (R) between adjacent loci is given at the right of the figure, with the standard error for each R.

Moreover, keratinocyte-specific post-translational modifications of the enzymes such as phosphorylation, glycosylation or non-covalent interactions with activating proteins cannot be excluded at present.

The genes of all mammalian lipoxygenases analyzed so far are organized in the same 14 exon format with exon/intron boundaries at highly conserved positions. The gene encoding mouse 12(*R*)-lipoxygenase, spanning approximately 12.5 kb, contains introns varying in length between 157 bp and 4.5 kb in exactly corresponding positions. With the exception of the unusual large intron 2, the exon clustering is similar to that found in the genes of the leukocyte type 12- and 15-lipoxygenases [12]. One major difference between the gene structures of *Alox12b* and the mammalian *S*-lipoxygenases is the presence of an additional intron (4a) which interrupts the sequence encoding the characteristic additional proline-rich stretch of 31 amino acids. So far the organization into 15 exons seems to be a unique feature of the *Alox12b* gene.

The mouse 12(*R*)-lipoxygenase chromosomal locus *Alox12b* is located to the central region of chromosome 11. The genes encoding the murine 12(*S*)-lipoxygenase isoforms of the platelet type (*Alox12*, formerly *Alox12p*), the leukocyte type (*Alox15b*, formerly *Alox12l*) and of the epidermis type (*Aloxe*) have been mapped to approximately the same central region of chromosome 11, while the human 12- and 15-lipoxygenase genes have been assigned to the syntenic region of human chromosome 17. The close chromosomal localization of 12- and 15-lipoxygenase genes suggests that they belong to a multigene family derived from a common ancestor gene. 12(*R*)-lipoxygenase, however, may represent a member of a distinct subfamily, the genes of which have acquired additional exonic and intronic sequences after gene divergence.

The *Alox12b* promoter lacks the CCAAT and TATA sequences. In this aspect, mouse 12(*R*)-lipoxygenase resembles most closely the 5-lipoxygenases from human and guinea pig [22,30] which also lack these elements, whereas the promoters of various 12- and 15-lipoxygenases contain a conserved TATA-like motif [23–25]. Although relatively G+C rich, the putative *Alox12b* promoter region did not reveal 100% matches to the Sp-1 consensus binding site found frequently in the promoter regions of all other mammalian lipoxygenases. The presence of repetitive sequences further upstream resembles some similarity with the putative promoter regions of the genes encoding the mouse platelet type and leukocyte type 12(*S*)-lipoxygenases both of which have been shown to contain highly repetitive sequences [23]. Consensus sequences to various *cis*-acting regulatory elements are found in the putative promoter region, among them binding sites for AP-2 which are also present in the promoter regions of 5-, platelet type 12- and 15-lipoxygenases [22–25,31]. Besides that, we found binding sites for GATA proteins and for transcriptional silencer which have been identified in the 5′ flanking region of rabbit and human 15-lipoxygenase [24,25]. To elucidate the functional significance of these elements for promoter activity and tissue-specific expression patterns, more detailed promoter/reporter gene expression studies are required.

5. Note added in proof

While this paper was in review, Sun et al. [32] described the cDNA cloning of the human and mouse 12(*R*)-lipoxygenase. The human enzyme was shown to metabolize arachidonic acid to 12(*R*)-HETE, whereas no enzymatic activity could be attributed to the murine ortholog.

Acknowledgements: The excellent technical assistance of Ina Kutschera, Brigitte Steinbauer and Andrea Pohl-Arnold is gratefully acknowledged. This work was supported by a grant of the Deutsche Krebshilfe.

References

[1] Spector, A.A., Gordon, J.A. and Moore, S.A. (1988) Prog. Lipid Res. 27, 271–323.
[2] Kühn, H., Belkner, J., Zaiss, S., Fahrenklemper, T. and Wohlfeil, S. (1994) J. Exp. Med. 179, 1903–1911.
[3] Feinmark, S.J. and Cornicelli, J.A. (1997) Biochem. Pharmacol. 54, 953–959.

[4] Shannon, V.R., Stenson, W.F. and Holtzman, M.J. (1993) Am. J. Physiol. 264, G104.
[5] Israel, E., Cohn, J., Dube, L. and Drazen, J.M. (1996) JAMA 275, 931–936.
[6] Ruzicka, T. and Printz, M.P. (1984) Rev. Physiol. Biochem. Pharmacol. 100, 121–160.
[7] Fogh, K., Kiil, J., Herlin, T., Ternowitz, T. and Kragballe, K. (1987) Arch. Dermatol. Res. 279, 504–511.
[8] Honn, K.V., Tang, D.G., Gao, X., Butovich, I.A., Liu, B., Timar, J. and Hagmann, W. (1994) Cancer Metastasis Rev. 13, 365–396.
[9] Krieg, P., Kinzig, A., Ress-Löschke, M., Vogel, S., Vanlandingham, B., Stephan, M., Lehmann, W.-D., Marks, F. and Fürstenberger, G. (1995) Mol. Carcinog. 14, 118–129.
[10] Radmark, O. (1995) J. Lipid Mediators. Cell Signal. 12, 171–184.
[11] Kühn, H. and Thiele, B.J. (1995) J. Lipid Mediators. Cell Signal. 12, 157–170.
[12] Yamamoto, S., Suzuki, H. and Ueda, N. (1997) Prog. Lipid Res. 36, 23–41.
[13] Brash, A.R., Boeglin, W.E. and Chang, M.S. (1997) Proc. Natl. Acad. Sci. USA 94, 6148–6152.
[14] van Dijk, K.W., Steketee, K., Havekes, L., Frants, R. and Hofker, M. (1995) Biochim. Biophys. Acta 1259, 4–8.
[15] Kinzig, A., Fürstenberger, G., Bürger, F., Vogel, S., Müller-Decker, K., Mincheva, A., Lichter, P., Marks, F. and Krieg, P. (1997) FEBS Lett. 402, 162–166.
[16] Jisaka, M., Kim, R.B., Boeglin, W.E., Nanney, L.B. and Brash, A.R. (1997) J. Biol. Chem. 272, 24410–24416.
[17] Krieg, P., Kinzig, A., Heidt, M., Marks, F. and Fürstenberger, G. (1998) Biochim. Biophys. Acta 1391, 7–12.
[18] Boeglin, W.E., Kim, R.B. and Brash, A.R. (1998) Proc. Natl. Acad. Sci. USA 95, 6744–6749.
[19] Krieg, P., Feil, S., Fürstenberger, G. and Bowden, G.T. (1993) J. Biol. Chem. 268, 17362–17369.
[20] Rowe, L.B., Nadeau, J.H., Turner, R., Frankel, W.N., Letts, V.A., Eppig, J.T., Ko, M.S.H., Thurston, S.J. and Birkenmeier, E.H. (1994) Mamm. Genome 5, 253–274.
[21] Yoshimoto, T. and Yamamoto, S. (1995) J. Lipid Mediators Cell Signal. 12, 195–212.
[22] Funk, C.D., Hoshiko, S., Matsumoto, T., Rdmark, O. and Samuelsson, B. (1989) Proc. Natl. Acad. Sci. USA 86, 2587–2591.
[23] Chen, X.-S., Kurre, U., Jenkins, N.A., Copeland, N.G. and Funk, C.D. (1994) J. Biol. Chem. 269, 13979–13987.
[24] O'Prey, J. and Harrison, P.R. (1995) Nucleic Acids Res. 23, 3664–3672.
[25] Kritzik, M.R., Ziober, A.F., Dicharry, S., Conrad, D.J. and Sigal, E. (1997) Biochim. Biophys. Acta 1352, 267–281.
[26] Kühn, H., Belkner, J., Wiesner, R. and Brash, A.R. (1990) J. Biol. Chem. 265, 18351–18358.
[27] Takahashi, Y., Glasgow, W.C., Suzuki, H., Taketani, Y., Yamamoto, S., Anton, M., Kühn, H. and Brash, A.R. (1993) Eur. J. Biochem. 218, 165–171.
[28] Kühn, H., Belkner, J., Suzuki, H. and Yamamoto, S. (1994) J. Lipid Res. 35, 1749–1759.
[29] Nugteren, D.H., Christ Hazelhof, E., van der Beek, A. and Houtsmuller, U.M. (1985) Biochim. Biophys. Acta 834, 429–436.
[30] Chopra, A., Ferreira Alves, D.L., Sirois, P. and Thirion, J.P. (1992) Biochem. Biophys. Res. Commun. 185, 489–495.
[31] Liu, Y.W., Arakawa, T., Yamamoto, S. and Chang, W.C. (1997) Biochem. J. 324, 133–140.
[32] Sun, D., McDonnell, M., Chen, X.-S., Lakkis, M.M., Li, H., Isaacs, S.N., Elsea, S.H., Patel, P.I. and Funk, C.D. (1998) J. Biol. Chem. 50, 33540–33547.