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M T Bassi, B Incerti, D J Easty, et al.

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LETTERS

Cloning of the Murine Homolog of the Ocular Albinism Type 1 (OA1) Gene: Sequence, Genomic Structure, and Expression Analysis in Pigment Cells

Maria Teresa Bassi,[1] Barbara Incerti,[2] David J. Easty,[3] Elena V. Sviderskaya,[3] and Andrea Ballabio[1,2,4]

[1]Department of Molecular Biology, University of Siena, Siena, Italy; [2] Telethon Institute of Genetics and Medicine (TIGEM), San Raffaele Biomedical Science Park, Milan, Italy; [3] St. George's Hospital Medical School, London SW17 0RE, UK

We report the isolation of the mouse homolog of OA1, the gene responsible for ocular albinism type I. The mouse Oa1 gene encodes a putative protein of 405 amino acids displaying a high level of homology (78% identity, 87% similarity) to the human gene. All disease-associated missense mutations reported in patients with ocular albinism involve conserved amino acid residues in the mouse protein. Moreover, the murine homolog shows six putative transmembrane domains, as observed for the human gene, indicating that the overall structure of the two proteins is conserved. The genomic organization is also conserved between the two species across the entire coding region with splice sites located in the same positions. Like its human counterpart, the expression pattern of Oa1, apart from the eye, is restricted to the epidermal melanocyte lineage. A transcript of ~1.8 kb was readily detected by this probe in 5 out of 5 murine melanocyte lines, 4 out of 4 murine melanoblast lines, 1 out of 2 murine melanoma lines, and 1 out of 2 human melanoma lines tested, but it was not detected in 2 out of 2 lines of a developmentally earlier normal cell type, melanoblast precursor cells, suggesting that the gene is transcriptionally activated in epidermal melanocytes at the same stage as most other tested melanosomal proteins. Together, these data suggest that the function of the OA1 gene is conserved between human and mouse and point to the mouse as a model to facilitate the understanding of ocular albinism pathogenesis.

Positional cloning efforts have led recently to the isolation of the human gene responsible for ocular albinism type 1, OA1 (MIM 300500), from the Xp22.3 region (Bassi et al. 1995). The disease is attributable to a pigmentation defect involving the melanocytes of the skin and of the retinal pigment epithelium. It is clinically characterized by severe reduction of visual acuity, photophobia, and iris translucency (O'Donnell et al. 1976; King et al. 1995). Electron microscopic analysis of the skin and eyes of affected individuals reveals the presence of macromelanosomes as large agglomerates of melanin in the melanocytes, supporting the hypothesis that the disease pathogenesis could involve one or more steps in the formation of melanosomes (O'Donnell et al. 1976; Garner and Jay 1980; Wong et al. 1983; Yoshiike et al. 1985).

The human OA1 gene spans 40 kb of genomic DNA (Schiaffino et al. 1995), is organized in nine exons, and is localized in a region of the human X chromosome that has been subject to recent evolutionary rearrangements (Palmer et al. 1995; Rugarli et al. 1995). Mutation analysis of the OA1 gene allowed us to identify mutations in only one-third of the patients studied (Schiaffino et al. 1995), even though linkage studies showed clearly that the disease is genetically homogeneous.

Sequence comparison of OA1 did not provide any valuable insight into the biochemical role of the OA1 gene product, which appears unrelated to molecules identified previously. Although the function of the OA1 protein product is unknown, recent data indicate that it is a mela-

[4]Corresponding author.
E-MAIL ballabio@tigem.it; FAX 39-2-21560-220.

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nosome-specific protein located on the melanosomal membrane, supporting the hypothesis of its involvement in melanosome biogenesis (Schiaffino et al. 1996). Here we report the isolation and sequencing of the murine Oa1 cDNA and the characterization of the complete genomic structure as the initial step toward developing a mouse model of the disease. We also report specific expression of Oa1 mRNA in cultured melanocytes, melanoblasts, and melanoma cells.

RESULTS AND DISCUSSION

Cross-species comparisons performed by Southern blot analysis revealed that OA1 was conserved in mouse and hamster (data not shown). To isolate the mouse homolog of the gene, a mouse retina cDNA library was screened using the full-length human OA1 cDNA as a probe. Two overlapping clones, MRIB and MR6, measuring 633 and 225 bp, respectively, were identified (see Fig. 1). Rescreening of the same library with the MRIB clone did not give any additional positive clones. To isolate the remaining portion of the cDNA, total RNA from a mouse melanoma cell line that expresses the gene was reverse-transcribed with oligo(dT) primers (see Methods) and the product was amplified using primers designed from the MRIB clone and from the sequence of a mouse genomic DNA fragment amplified by human exon 8-specific primers.

A PCR product of 727 bp was subcloned and sequenced (clone MOA13). The clone contained the region from nucleotide 446 to nucleotide 1173 of the cDNA. Experiments [3′ RACE (rapid amplification of cDNA ends)] were performed using the same reverse-transcription product mentioned above with oligo(dT) and a specific anchor primer based on the MOA13 clone (see Methods). A 281-bp fragment (57/5) was sequenced and shown to contain part of exons 8 and 9, including the translation stop codon. An additional round of amplification with a nested primer on exon 9 yielded the TV1 clone containing the remaining portion of the untranslated region and the poly(A) tail.

A contig of cDNA and PCR products was assembled, and the entire sequence was confirmed by sequencing the corresponding genomic clones. The sequence at the 5′ end of the MRIB clone (136 bp) appears to diverge from genomic DNA (clone 9N2 3.8; see Fig. 1) without showing the presence of any splice sites. Hybridization data indicate that this portion of the MRIB clone does not map to the OA1-related genomic region. Therefore, we assume that the divergence is owing to a rearrangement that occurred during the cDNA library construction at the 5′ end of the clone. Numbering of the nucleotide positions of the cDNA contig is such that position 1 corresponds to the first base that shows homology between cDNA and genomic sequences.

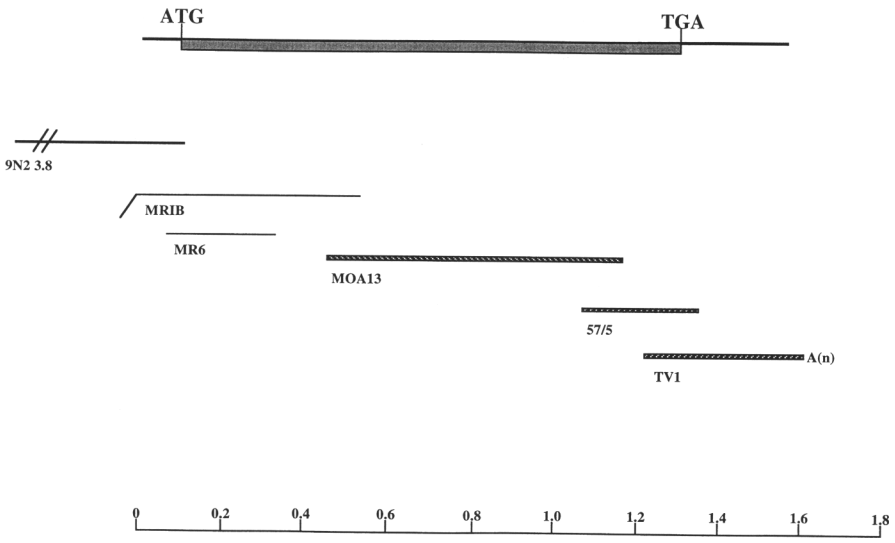

Figure 1 Schematic representation of the mouse Oa1 cDNA contig. The rectangle indicates the coding region, whereas the thin lines represent the 5′ and 3′ UTR, respectively. As explained in the text, the contig was assembled by using cDNA clones (thin lines) and PCR products (hatched bars). Solid bar (clone 9N2 3.8; not drawn to scale) represents a subclone of λ 9 genomic clone (Fig. 4) containing the first exon and the 5′ UTR of the gene (see text).

The cDNA contig measures 1613 bp with an open reading frame of 1215 bp encoding for a protein of 405 amino acids, very similar to the size of the human OA1 protein (404 amino acids). The first in-frame ATG codon in the mouse sequence is found at position 72 (exon 1) and corresponds to the second in-frame ATG in the human cDNA sequence. Although this ATG does not fulfill Kozak's criteria for being

an initiator codon, in-frame stop codons were found in the region upstream. The putative coding region ends with a TAG stop codon at position 1287 (exon 9) of the cDNA contig. High homology between the human and murine cDNA sequences starts right at the ATG, suggesting that this is the authentic start codon. Nevertheless, a low level of homology (61% of identity) is also detectable within 270 bp upstream of the ATG between the human and mouse genomic regions (data not shown). Figure 2 shows the alignment between the two proteins. Overall amino acid identity between OA1 and Oa1 is 78% with a similarity reaching 87%. As shown in Figure 2, the homology decreases in the carboxyl terminus corresponding to portions of exons 8 and 9.

Like the human counterpart, the hydrophobicity plot of the predicted mouse protein in Figure 3 indicates the presence of six hydrophobic regions, possibly corresponding to membrane-spanning domains. Sequence homology and protein structure data support the view that the general function of these two proteins is conserved (Figs. 2 and 3). Furthermore, all disease-associated missense mutations that were found in OA1-affected individuals involve conserved residues, suggesting that these amino acids play a critical role in the protein activity.

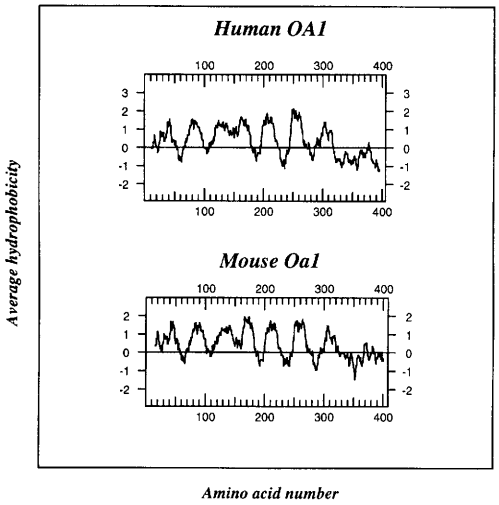

Figure 3 Hydrophobicity plot of the mouse (*bottom*) and human (*top*) proteins using the Kyte–Doolittle algorithm (Kyte and Doolittle 1982). Average hydrophobicity of a nonadecapeptide composed of amino acids $n - 9$ to $n + 9$ is plotted against n, the amino acid number. Six putative transmembrane domains (considering a score >1.6 in a segment of 19 amino acids) are observed.

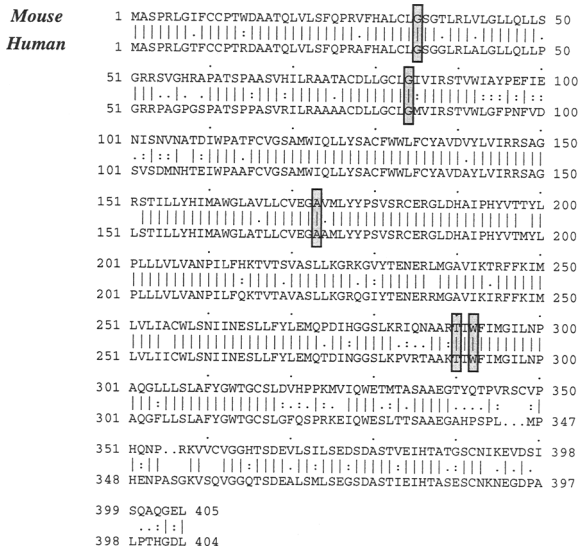

Figure 2 Comparison of the predicted protein products encoded by the human OA1 (*bottom*) and the mouse Oa1 (*top*) genes. Vertical bars indicate identical amino acids. Amino acids whose comparison value is ≥0.5 are marked with a colon; amino acids whose comparison value is ≥0.1 are indicated with a period. Shaded boxes indicate amino acids which are targets of point mutations (positions 35, 84, 173, and 292) and deletion (position 290) in OA1 patients.

Conservation between the human and mouse genes extends to the genomic organization that was determined by the isolation of four independent clones from a genomic library of mouse strain 129/SvEv in Lambda Fix II vector, as shown in Figure 4. The fragments identified by the cDNA were subcloned and sequenced both to confirm the cDNA sequence and to determine the exon–intron boundaries. The structure of the mouse Oa1 gene was found to be identical to that of the human gene, with nine exons and identical positions of all exon–intron junctions. The intron sizes in the mouse gene were smaller compared with the human homolog (Fig. 4). The extent of the genomic region covered by the Oa1 gene cannot be determined precisely because we could not obtain overlapping clones covering the entire length of intron 8. However, considering the smaller sizes of all mouse introns, we assume that the entire Oa1 gene spans ~20–25 kb, which is half the size of the human gene (Schiaffino et al. 1995).

Interestingly, the genetic mapping location

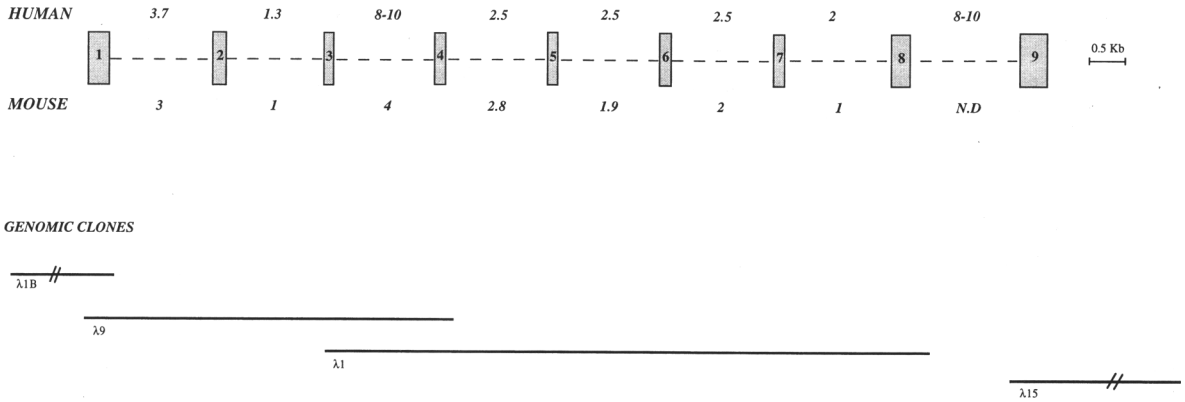

Figure 4 Genomic structure of the mouse gene. Exons represented as shaded boxes are drawn to scale as indicated in the figure. Numbers flanking the exons indicate the size of introns in the human gene (*top*) and in the murine homolog (*bottom*). The size of the intron between exons 8 and 9 was not determined (N.D.). The genomic clones (not drawn to scale) covering the Oa1 gene are shown on the *bottom*.

of Oa1 on the mouse X chromosome revealed a location between Alas2 and DXPas1, thus detecting a novel rearrangement that apparently occurred during recent mammalian X-chromosome evolution (Dinulos et al. 1996).

Apart from the eye, the expression of the mouse homolog appears to be confined to the epidermal melanocyte lineage. Northern analysis of RNA from various murine organs, a thymoma cell line RMA, and a murine keratinocyte line (XB2) revealed no hybridization with an Oa1 cDNA probe. However, a transcript of ~1.8 kb was detected readily by this probe in 5 out of 5 murine melanocyte lines, 4 out of 4 murine melanoblast lines, 1 out of 2 murine melanoma lines, and 1 out of 2 human melanoma lines tested. Sample results are shown in Figure 5. The expressing melanoma lines, B16 (data not shown) and RPMI 7932 (Fig. 5), are relatively well-differentiated. It is interesting that Oa1 expression was not detected in 2 out of 2 lines of a developmentally earlier normal cell type, melanoblast precursor cells (Fig. 5). Thus, the gene appears to be transcriptionally activated in epidermal melanocytes at the same stage as most other tested melanosomal proteins, including the TRP family and Pmel-17 (Sviderskaya et al. 1996).

Studies of human disease gene homologs from other species have provided valuable clues into the functional and biochemical features of the encoded proteins. The identification of evolutionary conserved regions between human and mouse OA1 may reflect the importance of preserving functionally critical domains. In particular, the mouse provides an opportunity to char-

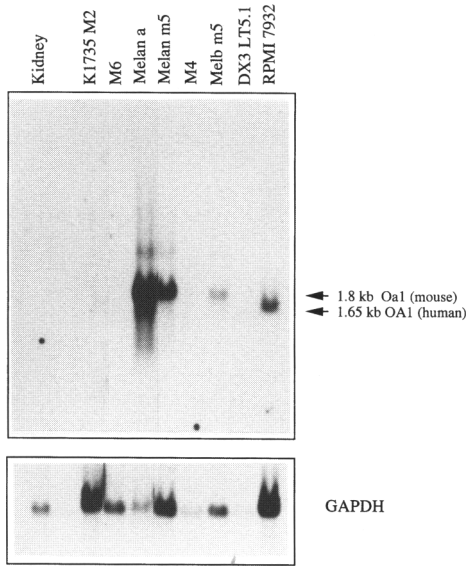

Figure 5 (*Top*) Specific expression of Oa1 mRNA in cultured melanocytes, melanoblasts, and melanoma cells. The autoradiograph of one of two blots of multiple melanocytic and other lines is shown. This blot contains poly(A)$^+$-enriched RNA from normal mouse kidney, a mouse melanoma line (K1735 M2), two melanocyte lines (melan-a, melan-m5), one melanoblast line (melb-m5), two lines of melanoblast precursors (see text), m4 and m6, and two human melanoma lines (DX3-LT5.1 and RPMI 7932). The arrows indicate the two different transcripts in human (1.65 kb) and in mouse (1.8 kb). (*Bottom*) The same blot was hybridized with a probe for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to assess the loading, and the 1.3-kb transcript is shown.

acterize the role of this gene and the events leading to the disease phenotype. Developing an animal model for the disease could aid in the dissection of the role of OA1 in the pathogenesis of ocular albinism.

METHODS

Isolation of Murine OAI cDNA and Genomic Clones

The full-length OA1 cDNA (1.6 kb) was used as a probe on an oligo(dT)-primed mouse retina cDNA library in λ ZAP vector (kindly provided by Dr. W.B. Baehr, Baylor College of Medicine, Houston, TX). One million independent clones were plated and transferred on Hybond-N (Amersham) nylon filters. Hybridization was carried out overnight in 6× SSC, 5× Denhardt's solution, 0.5% SDS, 100 μg/ml of salmon sperm DNA at 60°C. Filters were washed in 2× SSC, 0.1% SDS at 60°C. Recovery of λ cDNA clones in plasmids was performed using the λ ZAP plasmid rescue procedure according to the manufacturer's specifications.

RACE–PCR was performed on mouse melanoma cell line cDNA as described (Frohman et al. 1988). Briefly, 5 μg of total RNA from mouse melanoma cell line was reverse-transcribed with oligo(dT) primer using a kit from GIBCO BRL. The product was amplified successively with an adapter–oligo(dT) primer and a specific primer, moa4 in exon 2 (5′-TGCAACAGACATTTGGCCTGC-3′). Reamplification of the PCR product with two specific primers, moa1 in exon 3 (5′-GTTGTACAGTGCCTGCTTCTG-3′) and moa3 in exon 8 (5′-CTTCGTCAGAAGTCTGTCCCC-3′), led to the isolation of the MOA13 clone. The same reverse-transcription product was amplified using an adapter–oligo(dT) primer and a specific primer, moa6 (5′-ATGGGAATACTGAATCCAGCC-3′) in exon 8, designed on clone MOA13. The product was diluted 1:20 in TE and reamplified using the adapter primer and a nested primer, moa2 (5′-TGACTGCCTCTGCTGCTGAGG-3′), leading to the isolation of the 57/5 clone. A successive round of amplification with a primer, ex9-5′(5′-CAGATGCCAGTACTGTTGAAATCCA-3′), designed internally to moa2 gave the clone TV1 containing the poly(A) tail.

Plaques (6×10^5) of a mouse genomic library in λ FixII (Stratagene) were hybridized with full-length human cDNA. Rescreening of the library was carried out using two PCR products corresponding to the mouse exons 1 and 9 as probes. Hybridization was performed in standard conditions (Sambrook et al. 1989). Washes were carried out in 0.5× SSC, 0.1% SDS at 65°C.

Sequence and Expression Analysis

cDNA and genomic clone sequencing was performed either manually, using a Sequenase version 2.0 7-deaza–dGTP DNA sequencing kit (U.S. Biochemical), or automatically, using an Applied Biosystems ABI 377 fluorescent sequencer. Sequence assembly and analysis were performed using AutoAssembler TM 1.4 (Applied Biosystems) and DNA Strider 1.2 programs (Marck 1988). Nucleotide and amino acid sequences were compared with the nonredundant sequence data bases present at the National Center for Biotechnology Information using the BLAST network service.

Northern blot containing poly(A)$^+$-enriched RNA from several mouse tissues (heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis) was purchased from Clontech. A second Northern blot was prepared by us with total RNA from a mouse melanoma cell line (B16) and a mouse thymoma cell line (RMA) (kindly provided by Dr. C. Rugarli). Northern blot analysis was performed according to standard procedures (Sambrook et al. 1989). For other blots, poly(A)$^+$-enriched RNA was isolated from lines of murine melanocytes, melanoblasts, and melanoblast precursors (Bennett et al. 1987; Bennett and Sviderskaya 1996; Sviderskaya et al. 1996), also from murine melanoma line K1735-M2 and human melanoma lines DX3-LT5.1 and RPMI 7932, kindly provided by Drs. I. Fidler (M.D. Anderson Cancer Center, Houston, TX), I. Hart (St. Thomas's Hospital, London, UK), and G. Moore (Colorado Oncology Foundation, Denver), respectively. Isolation of RNA and blotting procedures for these samples were as described previously (Easty et al. 1995). In situ hybridization analysis performed on E10.5 to E17.5 mouse embryos failed to detect a significant level of Oa1 expression.

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