The highly conserved defender against the death 1 (DAD1) gene maps to human chromosome 14q11-q12 and mouse chromosome 14 and has plant and nematode homologs

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Abstract We have cloned the cDNA encoding the mouse DAD1 (defender against apoptotic cell death) protein. While showing an expected high homology with the previously cloned human and *Xenopus* DAD1-encoding cDNAs, this sequence has striking homology to partial cDNA sequences reported from *O. sativa* (rice) and *C. elegans* (nematode), suggesting the existence of plant and invertebrate homologs of this highly conserved gene. The human and mouse DAD1 genes map to chromosome 14q11-q12 and chromosome 14, respectively. This mapping data supports and extends the previously reported similarities between human chromosome 14q and mouse chromosome 14.

Key words: Cell death; Apoptosis; Programmed cell death; Chromosome 14; DAD1

1. Introduction

A number of conserved genes and biological pathways are believed to mediate apoptotic cell death [1,2]. For example, the Bcl-2 family comprises a structurally related group of proteins that may function as either agonists or antagonists of cell death in organisms as diverse as the nematode *C. elegans* and humans [1]. To illustrate this, it has been shown that the human Bcl-2 gene can rescue a *C. elegans ced-9* mutation [3] and that the baculovirus p35 gene can prevent death in mammalian cells [4]. Arguably, other genes subserving an essential regulatory function in cell death or cell survival must also be widely and highly conserved during evolution. Although a number of natural mutations in cell death or survival genes in *C. elegans* have been instrumental in identifying components of the cell death pathway, relatively few phenotypes resulting from mutations in mammalian cell death genes are known. To facilitate the identification of diseases that may be caused by defects in cell death genes, it would be essential to identify the chromosomal loci of these genes.

The DAD1 (defender against apoptotic cell death) gene was found to be essential for cell survival in a hamster cell line [5]. Molecular cloning of human, hamster and *Xenopus* DAD1 has

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DAD1 and *Dad1* are the approved symbols for the human and mouse gene, respectively; DAD1 refers to the protein products of these genes. The nucleotide sequence data described in this paper have been submitted to GenBank with Accession No. U22107.

demonstrated the remarkable degree of conservation of this protein in these 3 species [5]. It is identical in humans and hamster and varies only slightly in *Xenopus*. No homologies with previously isolated genes have been reported, and neither the precise intracellular localization nor the protein–protein interactions of DAD1 are currently known [5].

We report the cloning of the mouse *Dad1* cDNA. As expected, this is similar to the previously cloned human and hamster cDNAs. However, a search of the biological databases reveals the existence of related genes in *C. elegans* and *O. sativa* (rice), extending the conservation of this gene to invertebrates and plants. Furthermore, we demonstrate that the human and mouse DAD1 genes are located on chromosome 14 in both species.

2. Materials and methods

The sense primer 5'ATGTCGGCGTCGGTAGT3' (translation initiation codon underlined) and anti-sense primer 5'TCAGCCAACGAAGTTCAT3' (translation termination codon underlined) were used for the polymerase chain reaction (PCR) with cDNA from the K562 human erythroleukemia cell line to generate a *DAD1* probe. The same primers were used with newborn mouse skeleton cDNA as a template in order to clone *Dad1* cDNA. A PCR product of the expected size (340 bp) was found to hybridize to the *DAD1* cDNA and was subcloned into the plasmid pT7blue (Novagen, USA), to generate pm*Dad1*. Both strands of the cDNA were sequenced fully by automated sequencing. DNA sequences were analyzed using DNASTAR software programs.

For human chromosomal mapping, in situ hybridization was carried out on chromosome preparations obtained from phytohemagglutinin-stimulated lymphocytes cultured for 72 h. 5-Bromodeoxyuridine was added for the final several hours of culture (60 μg/ml of medium), to ensure a post-hybridization chromosomal banding of good quality. The PCR amplified *DAD1* clone was tritium labeled by nick-translation to a specific activity of 2.9×10^8 dpm/μg and hybridized to metaphase spreads at a final concentration of 100 ng/ml of hybridization solution as previously described [6]. After coating with nuclear track emulsion (Kodak NTB-2), the slides were exposed for 20 days at +4°C, then developed. To avoid any slipping of silver grains during the banding procedure, chromosome spreads were first stained with buffered Giemsa solution and metaphases photographed. R-banding was then performed by the fluorochrome-photolysis-Giemsa (FPH) method and metaphases rephotographed before analysis.

In order to determine the chromosomal location of the *Dad1* gene, we analyzed a panel of DNA samples from an interspecific cross that has been characterized for over 500 genetic markers throughout the genome. The genetic markers included in this map span between 50 and 80 centi-Morgans (cMs) on each mouse autosome and the X-chromosome [7,8]. C3H/HeJ-*gld* and *Mus spretus* (Spain) mice and [C3H/HeJ-*gld* × *Mus spretus*]F₁ × C3H/HeJ-*gld* interspecific backcross mice were bred and maintained as previously described [9]. *Mus spretus* was chosen as the second parent in this cross because of the relative ease of detection of informative restriction fragment length variants

(RFLVs) in comparison with crosses using conventional inbred laboratory strains.

DNA isolated from mouse organs by standard techniques was digested with restriction endonucleases and 10 μg samples were electrophoresed in 0.9% agarose gels. DNA was transferred to Nytran membranes (Schleicher and Schuell Inc., Keene, NH), hybridized at 65°C and washed under stringent conditions, all as previously described [10]. Initially, DNA from the two parental mice [C3H/HeJ-*gld* and [C3H/HeJ-*gld* × *Mus spretus*]F₁] were digested with various restriction endonucleases and hybridized with the [³²P]dCTP labeled *Dad1* probe to determine RFLVs to allow haplotype analyses. Informative *Eco*RI RFLVs were detected: C3H/HeJ-*gld*, 3.4 kb and 4.6 kb; *Mus spretus* 13.0 kb. Gene linkage was determined by segregation analysis [11].

3. Results and discussion

The sequence of pm*Dad1* was highly similar to published DAD1 sequences from human, hamster and *Xenopus*. The mouse DAD1 protein sequence is identical to the human and hamster sequences save for two residues, both of which are alanine-to-glycine substitutions (Fig. 1). The nucleotide sequence similarity is slightly less (94% and 91% identity to hamster and human sequences, respectively), resulting primarily from substitutions at the third bases of corresponding codons.

A search of the database of expressed sequence tags at the NCBI using the BLAST network service [12] revealed similarity to nucleotide sequences from *C. elegans* and *O. sativa* genomes (Fig. 1). The identity with the *O. sativa* sequence is 64% and 71% at the nucleotide and protein level, respectively, whereas the identity with the *C. elegans* sequence is 65% and 56%, respectively. If isofunctional amino acid substitutions are taken into account, then the available *O. sativa* and *C. elegans* sequences have an 88% and 81% similarity, respectively, with the mouse DAD1 protein sequence. Based on this high degree of

similarity of these sequences from evolutionarily distant species, we believe that these cDNAs encode the *C. elegans* and *O. sativa* counterparts of DAD1; the possibility also exists that these may be nematode and plant homologs of another gene closely related to DAD1.

In 100 metaphase cells examined after in situ hybridization for mapping of the human DAD1 gene, there were 208 silver grains associated with chromosomes and 44 of these (21.1%) were located on human chromosome 14; the distribution of grains on this chromosome was not random as 33/44 (i.e. 75%) mapped to the q11-q12 region. These results allow us to map the *DAD1* gene to the 14q11-q12 region of the human genome (Fig. 2a). A recent report on the composition of human chromosome 14 [13] and the Genome Database at Johns Hopkins University place several other gene loci in the vicinity of the *DAD1* gene. Hazan et al. [14] have mapped a neurodegenerative disease, autosomal dominant familial spastic paraplegia, to 14q11.2-q24.5. It is possible that this or other diseases could result from mutations in the *DAD1* locus.

For mapping of the *Dad1* gene, we used interspecific backcross analysis. Comparison of the haplotype distribution of the *Dad1* RFLV indicated that this gene co-segregated in 112 of 114 meiotic events with the *Top1rs1* locus on mouse chromosome 14 (Fig. 2b). Gene order was determined by analyzing all haplotypes and minimizing crossover frequency between all genes that were determined to be within a linkage group. This method resulted in determination of the most likely gene order [15]. The best gene order [15] ± the standard deviation [11] indicated the gene order: [centromere] *Top1rs1* − 1.8 cM ± 1.2 cM − *Dad1* − 8.8 cM ± 2.7 cM − *Rb1*. Previous studies in this interspecific cross have defined the location of the following reference loci used in these studies: topoisomerase 1 related sequence
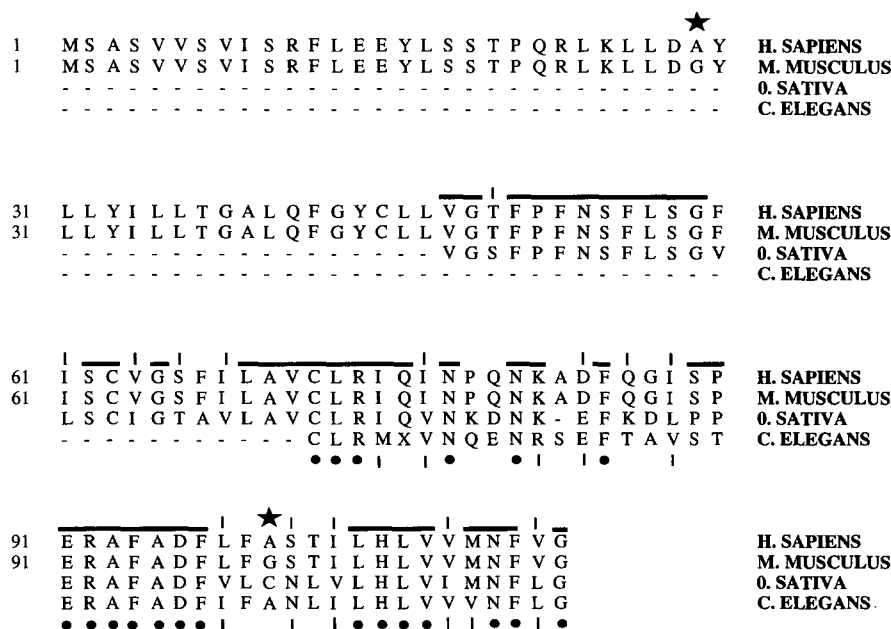

Fig. 1. Evolutionary conservation of the DAD1 gene. Alignment of the human (GenBank Accession No. S65756) and mouse DAD1 (GenBank Accession No. U22107) predicted amino acid sequences with sequences predicted by cDNAs from *O. sativa* (rice root, Genbank Accession No. D24136 (dbest)) and *C. elegans* (early embryo, GenBank Accession No. T01835 (dbest)). Since the latter two sequences are incomplete at their 5′ ends, only C-terminal peptide regions are available and the complete extent of similarity with DAD1 is not known. Two variant amino acid residues between human and mouse DAD1 are indicated by the asterisks. Residues that are identical between human, mouse, and rice sequences are indicated by the thick line above each alignment; short vertical lines indicate isofunctional residues. Residues that are identical or isofunctional in all four sequences are indicated by the filled circles and vertical lines, respectively, below each alignment. Numbering of amino acid residues is with reference to the human and mouse sequences. Sequences that are not available or a gap in the *O. sativa* sequence are also indicated (- - - -).
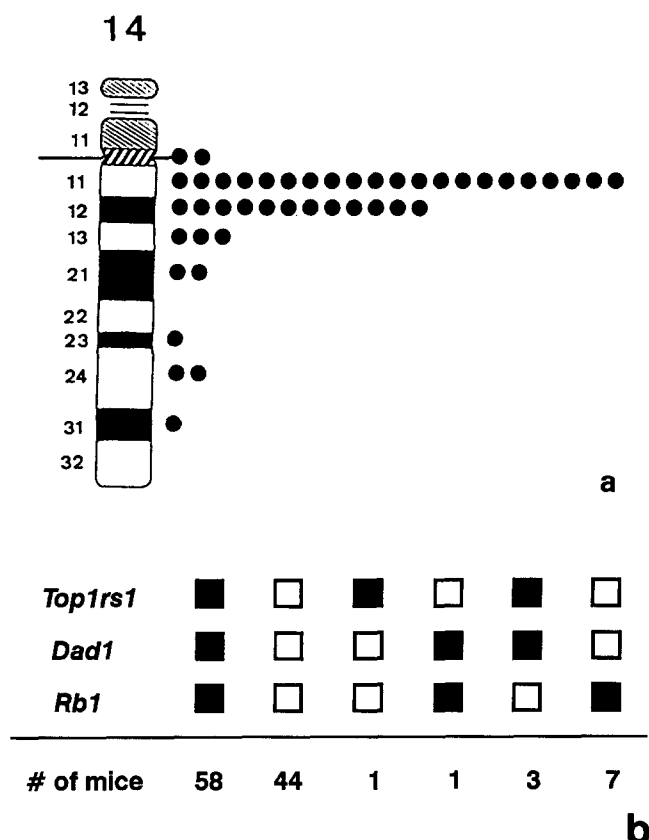
Fig. 2. Chromosomal mapping of the human and mouse DAD1 genes. (a) Idiogram of the human G-banded chromosome 14 illustrating the distribution of grains with the DAD1 probe. (b) Segregation of the *Dad1* locus on mouse chromosome 14 in [C3H/HeJ-*gld* × [C3H/HeJ-*gld* × *Mus spretus*]F₁] interspecific backcross mice. Filled boxes represent the homozygous C3H pattern and open boxes the F₁ pattern. The mapping of the reference loci in this interspecific cross has been previously described [16].

Although some invertebrate cell death genes such as the *C. elegans ced-3* and *ced-9* genes [1–3] have been shown to have mammalian homologs, our results show a dramatic conservation of a gene that is essential for cell survival in mammalian cells across plant, invertebrate and mammalian species. This underscores a possibly critical role for the DAD1 protein in maintenance of cell survival. Mapping of both the human and mouse DAD1 genes as we have reported here now makes possible studies to determine the role of this protein in both human and mouse inherited disease.

(*Top1rs1* [16], and the retinoblastoma gene 1 (*Rb1*) [16]. Consensus mapping places this gene at 33.3 cM since *Dad1* was 8.8 cM distal to *Ctla1* ([8], data not shown) and 8.8 cM proximal to *Rb1* that were positioned at 28.8 cM and 37.8 cM, respectively. This position is equally likely to correspond to human 13q14, 8p21-p11 or 14q based on composite comparative mapping data [17]. Mapping of the DAD1 gene indicates that this position corresponds to human 14q. Therefore, the current data helps clarify the comparative relationships between the two species.

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