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Isolation and structural characterization of a cDNA encoding *Arabidopsis thaliana* 3-hydroxy-3-methylglutaryl coenzyme A reductase

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Abstract

The enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (EC 1.1.1.34) catalyses the synthesis of mevalonate, the specific precursor of all isoprenoid compounds present in plants. We have characterized two overlapping cDNA clones that encompass the entire transcription unit of an HMG-CoA reductase gene from *Arabidopsis thaliana*. The transcription product has an upstream non-coding sequence of 70 nucleotides preceding an open reading frame of 1776 bases and a 3′ untranslated region in which two alternative polyadenylation sites have been found. The analysis of the nucleotide sequence reveals that the cDNA encodes a polypeptide of 592 residues with a molecular mass of 63 605 Da. The hydropathy profile of the protein indicates the presence of two highly hydrophobic domains near the N-terminus. A sequence of 407 amino acids corresponding to the C-terminal part of the protein (residues 172–579), which presumably contains the catalytic site, shows a high level of similarity to the region containing the catalytic site of the hamster, human, yeast and *Drosophila* enzymes. The N-terminal domain contains two putative membrane-spanning regions, in contrast to the enzyme from other organisms which has seven trans-membrane regions. *A. thaliana* contains two different HMG-CoA reductase genes (HMG1 and HMG2), as estimated by gene cloning and Southern blot analysis. Northern blot analysis reveals a single transcript of 2.4 kb in leaves and seedlings, which presumably corresponds to the expression of the HMG1 gene.

Introduction

Higher plants produce a vast array of isoprenoid compounds with a great variety of structures and functions [16]. Many isoprenoid compounds and derivatives play vital roles in plant metabolism and development. These include growth regulators (gibberellins and abscisic acid), side chains of many biologically active molecules (chlorophylls, prenylquinones and ubiquinone), carotenoids, dolichols and sterols. Some plants also produce and accumulate specific isoprenoid compounds

The sequence reported in this paper has been deposited in the EMBL/GenBank/DDBJ Nucleotide Sequence Databases under the accession number X15032.

with specific functions, such as phytoalexins for defence against microbial infection or fragrances that act as insect attractants for pollination.

Despite their wide diversity of structures and functions, all the isoprenoid compounds derive from a single common precursor, mevalonic acid. The synthesis of mevalonic acid is catalysed by the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (EC 1.1.1.34). The enzyme activity has been described for a large number of plants (for recent reviews, see ref. [3, 16]) and in all cases the enzyme is membrane-bound. The major subcellular location of the enzyme appears to be the endoplasmic reticulum, but the activity has also been reported to be associated with mitochondrial and plastid membranes [7, 31]. The occurrence of HMG-CoA reductase in different cellular compartments is a matter of controversy, and represents a major point to be clarified before an understanding of the control of isoprenoid metabolism in plants is reached.

HMG-CoA reductase has been extensively characterized in mammals in which it catalyses the major rate-controlling step in cholesterol biosynthesis. This control is achieved through complex processes at both transcriptional [26, 27] and post-transcriptional levels [9, 15]. Recently, cDNA and genomic HMG-CoA reductase clones have been isolated from *Drosophila* [14], yeast [5] and sea urchin [36], and from these the primary structure of the proteins has been deduced. It is now generally assumed that HMG-CoA reductase also catalyses the main rate-limiting step in plant isoprenoid biosynthesis, although more experimental data are needed to support this idea [16]. Despite the interest and relevance of plant HMG-CoA reductase very little is known about its structure and properties.

As a first approach to clone plant HMG-CoA reductase we chose *Arabidopsis thaliana*, since this plant provides an ideal model for plant genetics and molecular studies. Here we present data concerning the isolation and characterization of cDNA clones encoding *A. thaliana* HMG-CoA reductase.

Materials and methods

Plant material

Seeds of *A. thaliana* (Heyn), Columbia strain, were obtained from J.M. Martínez-Zapater and C.R. Somerville (MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI). Plants were grown under a 16 h light/8 h dark illumination regime at 22–24 °C on a perlite : vermiculite : sphagnum (1 : 1 : 1) mixture irrigated with mineral nutrients [34]. Axenic cultures were prepared by surface-sterilizing seeds in 5% sodium hypochlorite and germination on Petri dishes, containing mineral medium supplemented with 0.5% sucrose and 0.7% agar, under the conditions of light and temperature used for soil cultures.

Isolation and characterization of genomic and cDNA clones

An *A. thaliana* subgenomic library containing 10 to 14 kb *Bam* HI fragments was constructed in λ Charon 35 [23]. An *Eco* RI genomic library in *λsep6lac5* was obtained from E. Meyerowitz (California Institute of Technology, Pasadena, CA). A leaf λgt10 cDNA library from 4–5-week-old plants (rosette stage) was obtained from the laboratory of C.R. Somerville.

A Charon 35 recombinant clone (λgAT1) containing a 12.5 kb *Bam* HI genomic fragment was isolated using as a probe a 2.9 kb *Xba* I fragment from the hamster HMG-CoA reductase cDNA clone pRed-227 [8] (obtained from J.L. Goldstein and M.S. Brown, University of Texas Health Science Center, Dallas, TX). Hybridization and washings were performed under low-stringency conditions (35 °C below T_m). After restriction map analysis, the region responsible for hybridization to the hamster clone was located on a 2.8 kb *Xba* I-*Bam* HI fragment which was partially sequenced (Fig. 1).

A set of 12 independent cDNA clones (λcAT1 to λcAT12) was isolated from the *A. thaliana* leaf cDNA library using as a probe the 2.8 kb

Xba I-*Bam* HI fragment from λgAT1. Inserts contained in the recombinant phages were excised with *Eco* RI and subcloned into Bluescript plasmid (Stratagene, La Jolla, CA) for further analysis.

A set of three overlapping λ*sep6lac*5 recombinant phages (λgAT3, λgAT6 and λgAT10) was obtained after screening the genomic library with cDNA clone λcAT1 (Fig. 2) under moderate hybridization conditions (25 °C below T_m). The cloned region spans over 20 kb of genomic DNA, and contains a single transcription unit for HMG-CoA reductase (data to be published elsewhere).

DNA blot hybridization

Total DNA from 4–5-week-old rosette leaves was purified as described [11]. Restriction enzyme digestions were performed under the conditions recommended by the manufacturers and the resulting fragments resolved by agarose gel electrophoresis (TAE buffer). Alkaline transfer to Zeta-Probe membranes (Bio-Rad, Richmond, CA), hybridization with the probe and filter washes were performed according to the manufacturer's recommendations.

RNA analysis

Total RNA from either 4–5-week-old rosette leaves or 4–5-day-old seedlings was isolated as described [10], with minor modifications. Poly(A)$^+$ RNA was purified by oligo(dT) cellulose chromatography according to Aviv and Leder [2]. RNA samples were fractionated on 1% agarose/formaldehyde gels, transferred and UV-cross-linked to Zeta-Probe membranes [19]. Hybridization and washes were done as described [19]. The 5′ end of the mRNA was mapped by primer extension and S1 nuclease analyses [1]. For the primer extension analysis, 5 μg of of poly(A)$^+$ RNA was annealed to a ^{32}P-end-labelled synthetic 18-mer oligonucleotide (5′CCATTGGAGGGAATGAAT3′), complementary to the sequence comprising positions −14 to +4 of the cDNA sequence shown in Fig. 3. For the S1 nuclease mapping analysis, the same ^{32}P-end-labelled primer was annealed to a single-stranded template prepared from a recombinant plasmid containing a genomic fragment that extended 497 nucleotides upstream from the *Hae* III restriction site (GGCC) located at position +17 (Fig. 3). After elongation of the primer by the action of the Klenow fragment of *Escherichia coli* DNA polymerase I, the extended product was digested with *Rsa* I generating a 266 bp probe that was purified by alkaline agarose gel electrophoresis. The size of the S1 and the primer extension reaction products was estimated by polyacrylamide gel electrophoresis. A ladder of fragments generated by the chain-termination method (see below) using the same 18-mer primer was used as marker.

DNA sequencing

For DNA sequencing, the DNA fragments were subcloned into appropriate Bluescript plasmids. The DNA was sequenced by the dideoxy chain-termination method [32] modified by Biggin *et al.* [6] and using Sequenase™ (USB, Cleveland, OH).

Results

Isolation and characterization of a genomic fragment from A. thaliana *showing homology to a hamster HMG-CoA reductase cDNA clone*

The presence of DNA sequences in the genome of *A. thaliana* homologous to hamster HMG-CoA reductase was first demonstrated by Southern blot analysis. For this purpose, a 2.9 kb *Xba* I fragment, containing most of the hamster HMG-CoA reductase coding sequence, was isolated from the plasmid pRed-227 [8] and hybridized under low-stringency conditions (35 °C below T_m) to *A. thaliana* genomic DNA restriction frag-

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AT   1 N Y C S D K K A S A V N W I E G R G K H V V C E A F I K A E I V E K V L K T S V
HA 685 N Y C T D K K P A A I N W I E G R G K T V V C E A V I P A K V V R E V L K T T T

AT  41 E A L V E L N T L K N L V G S A M A G S L G G F N A H S S N I V S A V F I A T G
HA 725 E A M I D V N I N K N L V G S A M A G S I G G Y N A H A A N I V T A I Y I A C G

AT  81 Q D Q L Q N V E S S H C M T M I - - - L P D G D D L H I S V S M P C I E
HA 765 Q D A A Q N V G S S N C I T L M E A S G P T N E D L Y I S C T M P S I E
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Fig. 1. Sequence homology comparison between a segment of the protein encoded by the *A. thaliana* genomic clone λgAT1 (AT) and hamster HMG-CoA reductase (HA). The *A. thaliana* amino acid sequence was deduced from the nucleotide sequence of the region responsible for the hybridization to the hamster cDNA clone pRed-227. The hamster amino acid sequence shown corresponds to amino acid residues 685–800 of the published sequence [8]. The standard single-letter amino acid code is used. Common amino acids are indicated by solid boxes; conservative amino acid substitutions are indicated by stippled boxes. Only conservative substitutions having a value of 5 in the structural resemblance and genetic interconvertibility matrix reported by Doolittle [12] are considered.

ments. The results obtained revealed the presence of faint, but clearly distinguishable, hybridization bands (not shown). To further characterize the DNA regions responsible for the hybridization signals, the recombinant phage λgAT1 containing a 12.5 kb *Bam* HI fragment was isolated from an *A. thaliana* genomic library (see Materials and methods). The clone was analysed in detail and the region homologous to hamster HMG-CoA reductase was located within a 2.8 kb *Xba* I-*Bam* HI fragment which was partially sequenced. The obtained nucleotide sequence showed the presence of an open reading frame coding for an amino acid sequence that could be aligned to the region between residues 685 and 800 of the hamster enzyme (Fig. 1). Comparison of the sequences revealed extensive conservation (62% identical residues and 17% conservative changes) in a region corresponding to the catalytic site of the hamster enzyme [22].

Isolation and nucleotide sequence of A. thaliana *cDNA HMG-CoA reductase clones*

A leaf cDNA library from 4–5-week-old plants was screened using the 2.8 kb *Xba* I-*Bam* HI fragment from the genomic clone λgAT1 as a probe. Several positive clones were obtained and characterized by restriction enzyme mapping. Figure 2A shows the composite restriction map for two overlapping clones (λcAT1 and λcAT12) spanning 2373 bp. This value is in close agreement to

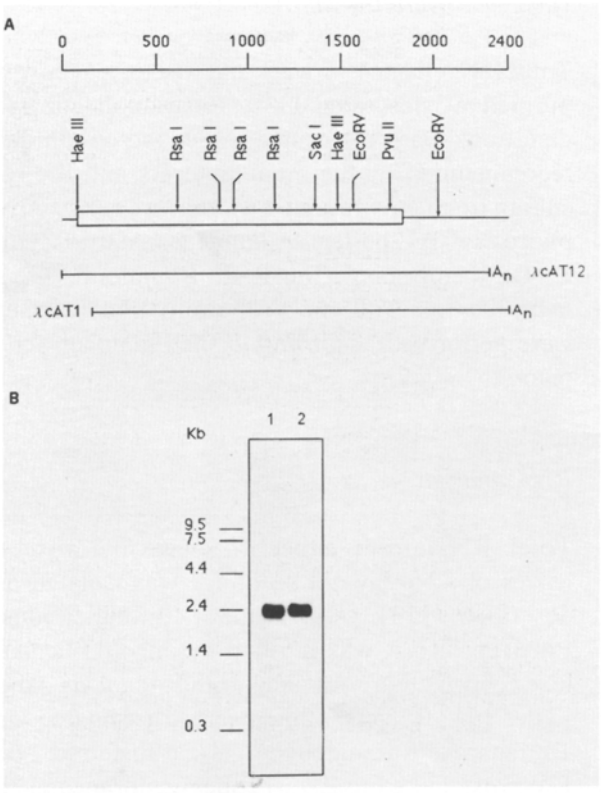

Fig. 2. A. Composite restriction map of the two overlapping *A. thaliana* HMG-CoA reductase cDNA clones λcAT1 and λcAT12. The boxed region indicates the coding sequence. B. Northern blot-hybridization of *A. thaliana* total RNA (3 μg/lane) from 4–5-week-old rosette leaves (lane 1) and 4–5-day-old seedlings (lane 2). The probe used was the λcAT1 cDNA insert. Mobility of molecular size markers is indicated. The scale on top is in bp.

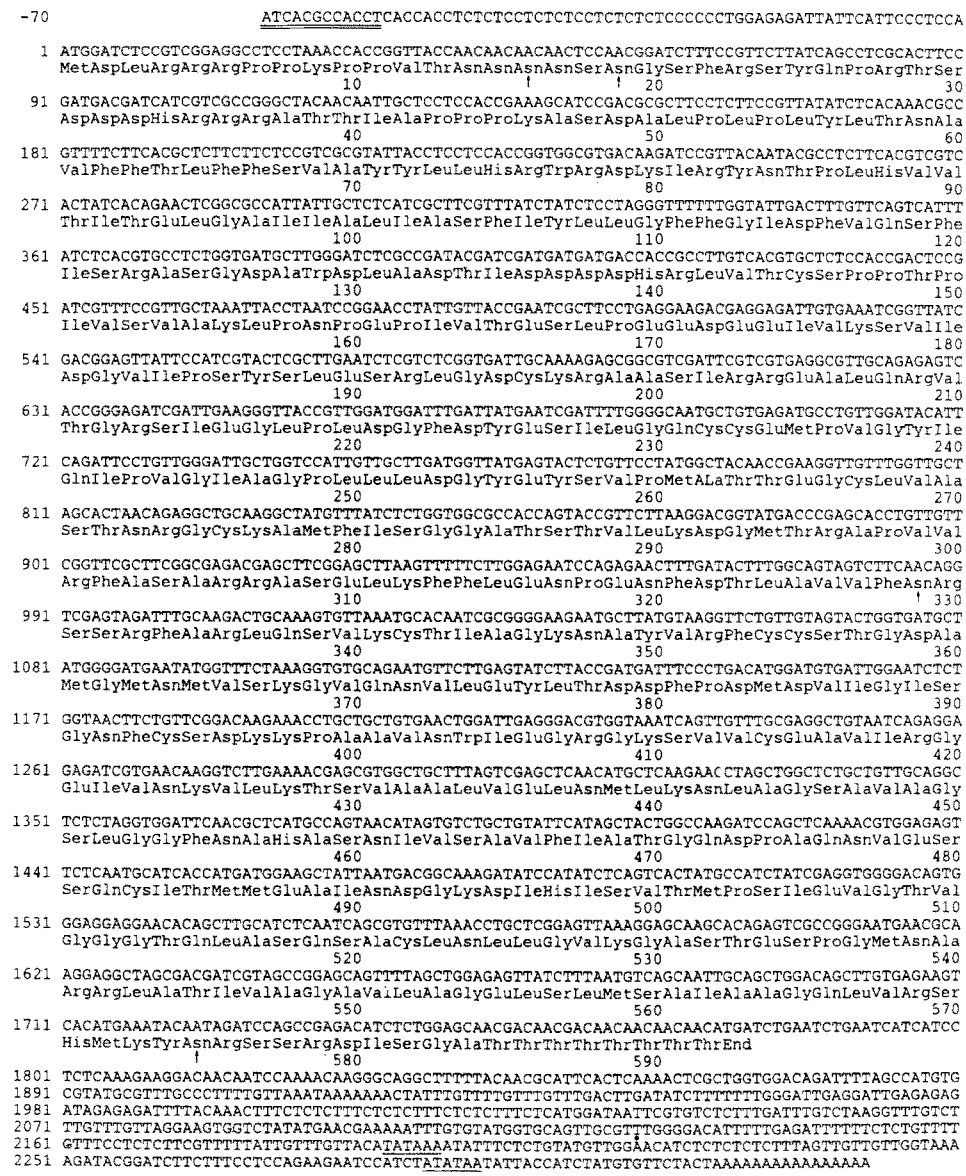

Fig. 3. Combined nucleotide and predicted amino acid sequences of cDNA clones λcAT1 and λcAT12 corresponding to *A. thaliana* HMG-CoA reductase. Both sequences are numbered with respect to the putative initiating methionine. The double-underlined sequence at the 5′ end was obtained from the corresponding genomic clone together with primer extension and S1 nuclease analyses data. The two putative alternative polyadenylation signals are underlined. The polyadenylation site in λcAT12 is indicated by a filled circle. Four potential N-linked glycosylation sites are marked with arrows.

the transcript size of 2.4 kb estimated by Northern blot analysis (Fig. 2B). The nucleotide sequence of this HMG-CoA reductase cDNA and the predicted amino acid sequence of the protein are shown in Fig. 3. The complete nucleotide sequence of the upstream non-coding sequence was obtained by sequencing the corresponding region in the genomic clone λgAT3, together with data from S1 mapping and primer extension analyses (Fig. 4). The transcript contains a 70 bp upstream non-coding sequence, preceding the first AUG codon starting an open reading frame of 1776 bases. The assignment of the initiating methionine was from the general observation that translation

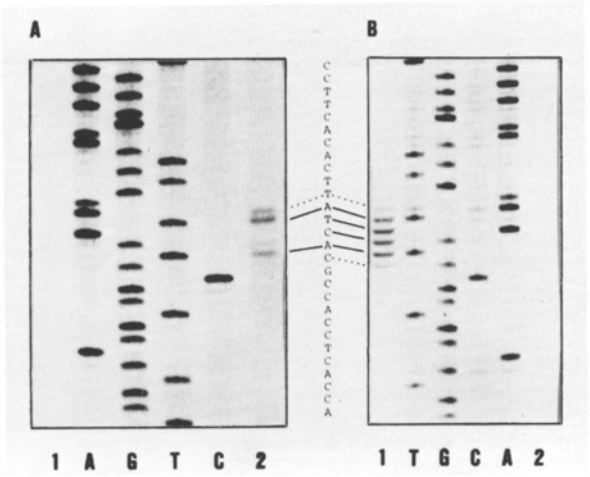

Fig. 4. Mapping of the 5′ end of *A. thaliana* HMG-CoA reductase. A. Primer extension. The ^{32}P-end-labelled 18-mer oligonucleotide described in Material and methods was hybridized to 5 μg of poly(A)$^+$ RNA and extended with reverse transcriptase (lane 2). No extended products were detected when the primer extension was performed in the presence of 5 μg of calf liver tRNA (lane 1). B. S1 nuclease protection. The 266 bp end-labelled probe described in Material and methods was hybridized to 5 μg of poly(A)$^+$ RNA and treated with S1 nuclease (lane 1). No protected fragments were detected in a control experiment performed in the presence of 5 μg of calf liver tRNA (lane 2). In both primer extension and S1 nuclease protection assays the reaction products were fractionated on a 10% polyacrylamide gel containing 8 M urea. The sequence ladder was obtained by using the 18-mer oligonucleotide as a DNA-sequencing primer on the appropriate template. The sequence surrounding the transcription start site is shown, and the location of the primer-extended and nuclease-protected products is indicated (solid and dotted lines).

of most eukaryotic mRNAs begins at the 5′ proximal AUG codon [20]. Furthermore, the sequence surrounding this AUG codon fits the consensus sequence (AACAAUGGC) reported for the initiation codon in plants [25]. Two alternative polyadenylation sites have been detected in the 3′ untranslated region, at positions +2218 and +2316, which are preceded by two putative polyadenylation signals at positions +2193 (TATAAA) and +2287 (ATATAA) respectively. This is in agreement with the consensus polyadenylation sequence (A/GAATAAPu) described in plants [18]. The sequencing of the homologous region in the genomic clone λgAT3 confirmed that the differences detected in the length of the 3′ end are the result of polyadenylation at different sites instead of an artefactual recombination event. The significance of the heterogeneity in the 3′ untranslated region has not been further pursued.

A. thaliana HMG-CoA reductase mRNA encodes a protein of 592 amino acids with a molecular weight of 63 605 Da. The hydropathy profile of the protein, deduced by the algorithm of Kyte & Doolittle [21], shows the presence of two hydrophobic regions located within the first 117 residues (residues 47–69 and 83–117), each of which is long enough to span a membrane bilayer (regions 1 and 2 in Fig. 5). These hydrophobic regions are separated and flanked by hydrophilic sequences rich in charged residues. Since it is well known that plant HMG-CoA reductase is a membrane-bound protein [3, 16], it is reasonable to postulate that these N-terminal hydrophobic regions could correspond to trans-membrane domains.

The protein contains four potential asparagine-linked glycosylation sites (Asn-X-Ser/Thr) at positions 16, 19, 329 and 575. Nevertheless, no data are presently available on the glycosylation of plant HMG-CoA reductase. The linker region joining the N- and C-terminal regions (see below) contains a typical 'PEST' sequence (residues 156–177) described to be present in proteins having rapid turnover rates [29].

Comparison of A. thaliana *HMG-CoA reductase to the enzyme from other organisms*

At present, the primary sequence of HMG-CoA reductase is known from a variety of organisms including hamster [8, 33], man [24], *Drosophila* [14] and yeast [5]. The partial amino acid sequence of the enzyme from sea urchin has also been reported [36]. From these data it becomes evident that all HMG-CoA reductases characterized so far share a high level of similarity in the C-terminal region, that contains the catalytic site. In this respect, the C-terminal part of the *A. thaliana* enzyme (residues 172–579) also

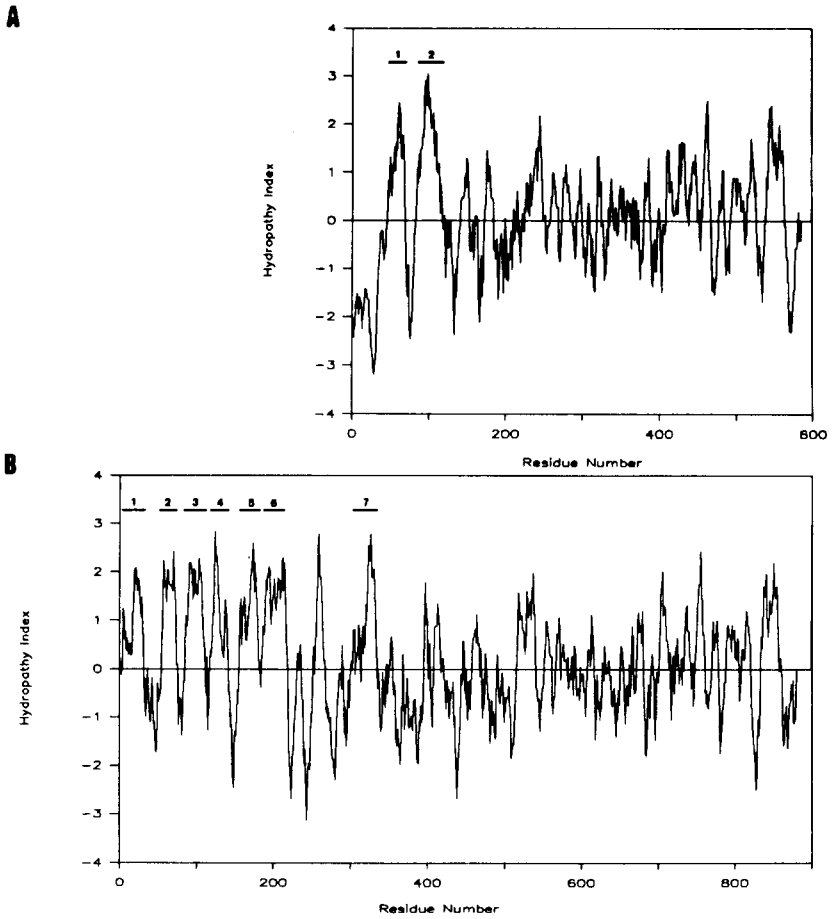

Fig. 5. Hydropathy index plot of the predicted *A. thaliana* (A) and hamster (B) HMG-CoA reductase. The algorithm of Kyte and Doolittle [21] was used with a window size of 9 residues. Positive values indicate hydrophobic regions. Bars 1 and 2 (A) and 1 to 7 (B) indicate probable membrane-spanning regions.

shows a high level of similarity to the previously reported HMG-CoA reductase sequences (Fig. 6), with values ranging from 56 to 58% identical and 12 to 14% conserved residues. In addition, the alignment shown in Fig. 6 reveals that 40% of the amino acids are identical and 16% of the changes are conservative in all proteins. Those amino acids located in identical position are of special interest since they can reveal interesting features concerning the functional and structural properties of the enzyme. In this respect, only two histidine and five cysteine residues (marked in Fig. 6) are common to all HMG-CoA reductases. In addition, all the enzymes compared share a strikingly high proportion of glycine residues located in the region containing the catalytic site. Their possible functional and structural implications will be discussed below.

One of the most intriguing differences between *A. thaliana* HMG-CoA reductase and the enzyme from other organisms is found in the N-terminal part of the protein. *A. thaliana* HMG-CoA reductase shows a remarkable different structure, with only two potential trans-membrane domains, in contrast with the structure of the equivalent region of the enzyme from mammals [8, 24, 33], *Drosophila* [14] and yeast [5], described to contain seven potential membrane-spanning domains. Moreover, the N-terminal region of the plant enzyme is much shorter and shows no significant homology to the membrane domain of the other HMG-CoA reductases.

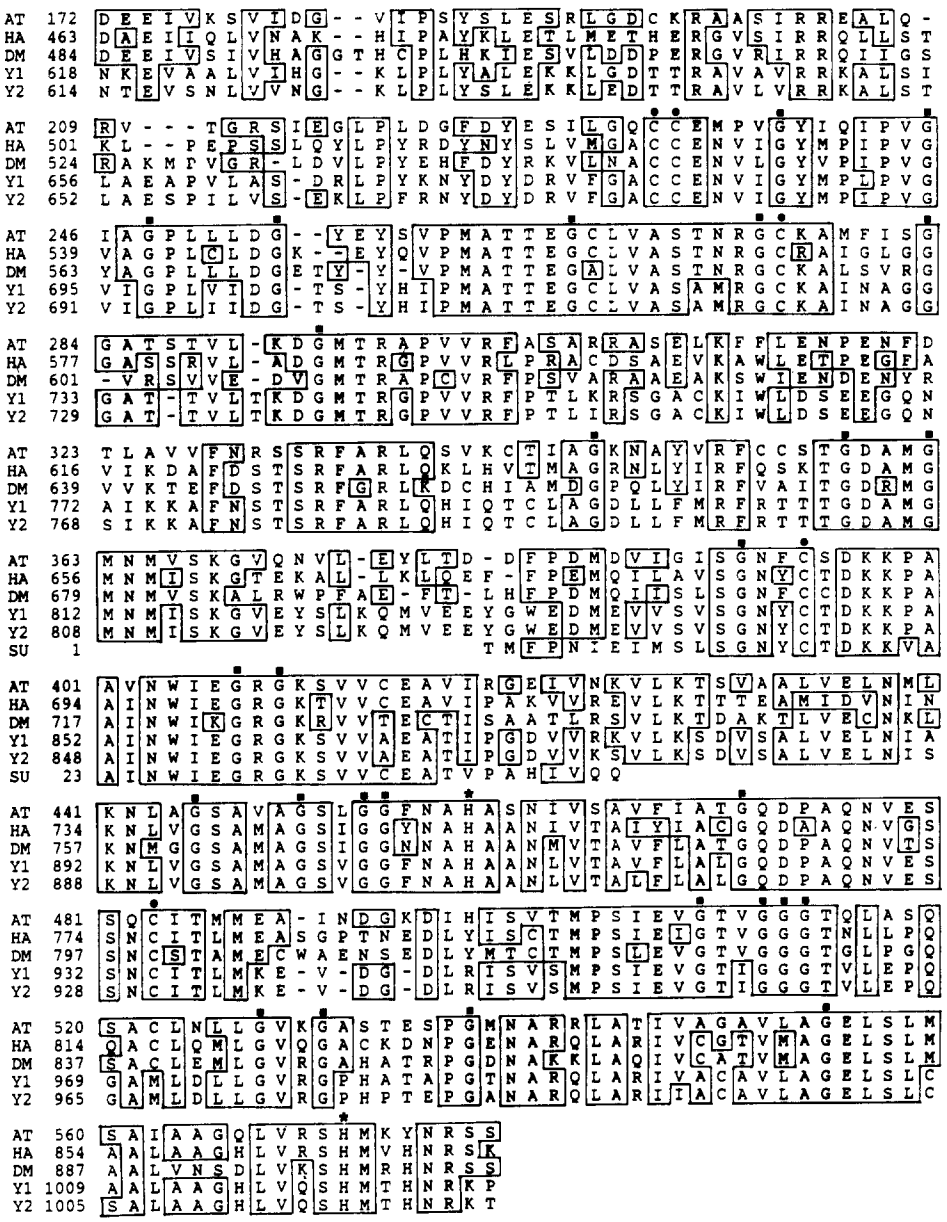

Fig. 6. Homology comparison of the predicted C-terminal amino acid sequence of *A. thaliana* HMG-CoA reductase (AT) with those from hamster (HA), *Drosophila* (DM), yeast HMG1 (Y1), yeast HMG2 (Y2) and sea urchin (SU). Gaps (hyphens) were introduced to optimize aligments. Only residues identical to the *A. thaliana* enzyme are boxed. Histidine (*), cysteine (●) and glycine (■) residues which are conserved in all proteins are marked (see text). Amino acids are shown in the single-letter code and numbered (with the only exception of sea urchin) from the N-terminal methionine of each protein. The region extending over the β-domains b1 and b2, reported to contain the catalytic site of the hamster enzyme [23], corresponds to residues 223 and 294 and 441 to 531 respectively in the *A. thaliana* enzyme.

The linker region, defined as the region between the last predicted membrane-spanning domain and the start of the sequence conserved among the different enzymes (residues 118–171, Fig. 3), is much shorter than the equivalent linker region of the enzyme from other organisms. No detectable sequence similarity has been found in the linker region of the different HMG-CoA reductases.

Arabidopsis thaliana *contains two genes coding for HMG-CoA reductase*

After sequencing of the cDNA clones isolated from the leaf cDNA library, it was clear that the primary sequence of the encoded protein was different from that first deduced from the genomic clone λgAT1 (Fig. 1). After restriction enzyme mapping of 12 independent cDNA clones obtained from this library, it was concluded that none of them were coded by the genomic clone λgAT1 first isolated, thus indicating that these cDNA clones corresponded to the product of a different gene. Recent data from our laboratory have confirmed that these cDNAs correspond to transcripts encoded by the gene present in the overlapping clones λgAT3 and λgAT6 (unpublished results).

The existence of two different genomic sequences in *Arabidopsis* coding for HMG-CoA reductase was confirmed by Southern blot analysis, using either the genomic clone λgAT1 or the cDNA clone λcAT1 as a probe. The pattern found clearly indicates that each probe identifies a different set of genomic fragments when the hybridizations are performed under moderate stringency conditions (Fig. 7). Under low-stringency hybridization conditions, or after long autoradiographic exposures, a mutually complementary pattern of bands can be observed, thus confirming that both genomic sequences are closely related (not shown).

Fig. 7. Genomic DNA blot-hybridization analysis. *A. thaliana* DNA (1 μg/lane) was digested with *Bam* HI (lanes 1), *Eco* RI (lanes 2), *Hin*d III (lanes 3) and *Xba* I (lanes 4). Fragments were electrophoretically fractionated, bound on a Zeta-Probe filter and hybridized with a ^{32}P-labeled probe bearing either the λcAT1 cDNA clone (A) or the λgAT1 genomic clone (B). Probes were, for the λcAT1 clone, the complete cDNA insert and, for the λgAT1 clone, the 2.8 kb *Xba* I-*Bam* HI fragment. DNA molecular size markers are indicated at the left.

Discussion

The strategy devised for cloning a plant HMG-CoA reductase gene was based upon the assumption that enzymes that catalyse key reactions in intermediary metabolism may be conserved during evolution. In the case of HMG-CoA reductase this hypothesis was supported by the results of Basson *et al.* [4], who reported that the enzymes from two distantly related organisms, such as hamster and yeast, were very similar in the region containing the catalytic site. From recent characterization of cDNA and genomic clones, it is now clear that the catalytic domain of HMG-CoA reductase is highly conserved between distantly related organisms such as *Drosophila* [14], yeast [5], sea urchin [36] and mammals [8, 24, 33].

As a first approach to clone plant HMG-CoA reductase, we tried to detect the presence of sequences homologous to hamster HMG-CoA reductase in the genome of *A. thaliana*. DNA blots clearly demonstrated the existence in the plant of sequences homologous to the hamster HMG-CoA reductase cDNA clone pRed-227 [8]. This was the starting point for the isolation of an *A. thaliana* genomic clone, which in turn was used as a probe to further isolate the cDNA clones characterized in the present work.

The amino acid sequence deduced from the nucleotide sequence of the cDNA clones (Fig. 2) reveals some interesting differential features of the

plant enzyme with respect to the other HMG-CoA reductases characterized so far. The most striking difference is its short N-terminal domain, with only two potential membrane-spanning regions. This is in contrast with the general occurrence of seven membrane-spanning regions in the rest of the organisms studied. Furthermore, no significant sequence similarity is detected between the membrane spanning domains of the *A. thaliana* enzyme and those of the enzyme from other organisms. This trans-membrane region is partially conserved between the hamster and the *Drosophila* enzyme, but not between hamster and yeast. At present, it is difficult to assess if the cloned enzyme represents the endoplasmic reticulum form as in plants the enzyme activity has also been reported to be associated to mitochondrial and plastid membranes [7, 31]. Although no clear consensus sequences have yet been defined for the transit peptides of plant mitochondrial and chloroplast proteins [35], some features of the N-terminal part of the protein, rich in charged and hydroxylated residues, suggest that the cloned enzyme could correspond to the plastid form. Nevertheless, further experiments are needed to clarify the intracellular location of the enzyme.

All HMG-CoA reductases, including the *A. thaliana* enzyme, share a great level of conservation of their C-terminal region (Fig. 6). This observation suggests that this domain probably contains the catalytic site of the plant enzyme, as postulated for the hamster enzyme [22], and that its structural conservation reflects its essential function. The alignment in Fig. 6 shows that 40% of the amino acid residues in the C-terminal domain are identical in all the enzymes, thus suggesting a strong evolutionary pressure to maintain these specific amino acids at defined positions. Some of these amino acids may play important roles in the structural conformation and/or in the catalytic properties of the enzyme. In particular, the most conserved region among the different HMG-CoA reductases lies in the β-domains (b1 and b2) that have been postulated to be part of the active site for the hamster HMG-CoA reductase [22]. This region is specially rich in cysteine and glycine residues. The conserved glycine residues may be important in the maintenance of the correct structure of the β-domains b1 and b2. Since it is also known that HMG-CoA reductase requires a high concentration of thiol-reducing agents for its activity [30], the conserved cysteine residues in this region (Fig. 6) may reflect their importance, not only for the appropriate conformation of the catalytic site of the enzyme, but also for its active role in the catalytic process as described by Dugan and Katiyar [13]. Out of the two conserved histidine residues, the one contained within domain b2 (Fig. 6) is the most likely to participate in the transient protonation reported to occur during the catalytic process [28]. It is also interesting to note the great similarity in the hydropathy profiles of the conserved C-terminal regions when analysed under the same parameters (not shown), indicating that the differences existing in the primary sequence of the proteins probably would not affect the overall structure of the catalytic domain.

Our results indicate that *A. thaliana* contains at least two different genes coding for HMG-CoA reductase. One of these genes, designated HMG1 according to the genetic nomenclature recently recommended for *Arabidopsis* [17], codes for the enzyme described in this paper. This gene is actively expressed in leaves and light-grown seedlings where it seems to represent the major, if not the unique, HMG-CoA reductase transcript. This conclusion is supported by the results of Northern blot experiments using the probes corresponding to HMG1 and HMG2 that were also used in the Southern blot experiments reported above (Fig. 7). In Northern blot analysis performed under moderate hybridization conditions (25 °C below T_m) both probes detect a single transcript of 2.4 kb. Nevertheless, under high-stringency conditions (10 °C below T_m) the transcript is only detected when the HMG1 probe is used (data not shown). Since under high-stringency hybridization conditions the probes behave as specific for each gene (shown in Fig. 7), we can conclude that HMG2 gene is probably not expressed in leaves and light-grown seedlings. However, further studies are needed to establish the relative pattern of expression of these genes

during plant development. It would also be interesting to know if the two genes code for different isoenzymes serving specific functions and having different intracellular locations.

Acknowledgments

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