ELSEVIER

Biochimica et Biophysica Acta 1350 (1997) 247–252

BIOCHIMICA ET BIOPHYSICA ACTA

BBA

Short sequence-paper

# Molecular cloning of ozone-inducible protein from *Pinus sylvestris* L. with high sequence similarity to vertebrate 3-hydroxy-3-methylglutaryl-CoA-synthase [1]

Andrea Wegener [a], Wolfgang Gimbel [b], Thomas Werner [c], Jean Hani [d], Dieter Ernst [a,*], Heinrich Sandermann Jr. [a]

[a] *GSF — National Research Center for Environment and Health, Institute of Biochemical Plant Pathology, D-85764 Neuherberg, Germany*

[b] *GSF — National Research Center for Environment and Health, Institute of Molecular Virology, D-85764 Neuherberg, Germany*

[c] *GSF — National Research Center for Environment and Health, Institute of Mammalian Genetics, D-85764 Neuherberg, Germany*

[d] *Genzentrum der LMU München, Würmtalstr. 221, D-81375 München, Germany*

Received 25 October 1996; accepted 31 October 1996

## Abstract

We have isolated a *Pinus sylvestris* cDNA encoding a globular protein of 474 amino acids with a predicted molecular weight of 52 995 Da. The deduced amino acid sequence showed 41.9% identity and 13.6% similarity to mammalian cytosolic 3-hydroxy-3-methylglutaryl-CoA-synthase (HMGS). Treatment of Scots pine seedlings with ozone resulted in a transient increase of a 1.95 kb transcript, whereas a 1.2 kb mRNA decreased transiently, indicating a possible influence of ozone on isoprenoid biosynthesis.

*Keywords:* Ozone; 3-hydroxy-3-methylglutaryl-CoA-synthase; cDNA; mRNA; (*Pinus sylvestris* L.)

Ozone is an important photochemical air pollutant and known to be toxic to plants [1]. At the molecular level, transcripts of defense-related genes are induced in herbaceous plants. This includes an accumulation of pathogenesis-related mRNAs, transcripts involved in phenylpropanoid pathway, and antioxidative transcripts [2–5]. Many of these responses have been summarized elsewhere [6,7]. Some of these responses are induced upon wounding, pathogen attack and UV irradiation [8–10]. In contrast to herbaceous plants, little is known about ozone effects at the molecular level in conifers. Ozone treatment of Norway spruce seedlings resulted in an accumulation of cinnamyl alcohol dehydrogenase (CAD) mRNA [11]. CAD is an important enzyme of lignification, which is also induced upon fungal attack [12]. A systematic search for Scots pine genes that are activated upon ozone treatment, using in vitro translation studies, resulted in several ozone-induced, as well as ozone-repressed transcripts [13]. Recently extensin mRNA was shown

---

[*] Corresponding author. Fax: +49 89 31873383; E-mail: ernst@gsf.de

[1] The nucleotide sequence data reported in this paper have been submitted to the EMBL Databank under the accession number X96386.

*PII* S0005-2760(96)00161-0

to be accumulated upon ozone fumigation in Scots pine, Norway spruce and European beech [14].

In plants HMGS catalyzes the condensation of acetyl-CoA with acetoacetyl-CoA, resulting in the formation of 3-hydroxy-3-methylglutaryl-CoA. This compound is reduced to mevalonic acid, a precursor of isopentenyl diphosphate [15]. Isopentenyl diphosphate, named also 'active isoprene unit' is the basic precursor for the synthesis of isoprenoids, important secondary products of higher plants [16]. The biosynthesis of isoprenoids is complex, however, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) is thought to be a key enzyme of isoprenoid biosynthesis [17,18]. Aside developmental regulation, isoprenoids can be specifically induced by wounding, pathogen attack or elicitor treatment (reviewed in [19]). HMGR transcripts have shown to be increased upon such a biotic stress [20,21]. In contrast to HMGR, HMGS has not been studied extensively in plants (reviewed in [19]). In animals it has been shown that the rate-limiting reaction in cholesterol biosynthesis is catalyzed by HMGR (reviewed in [22]). However, transcript accumulation of HMGS and HMGR is coordinately regulated by cholesterol [23,24]. Although HMGS activity has been demonstrated in plants, no purified enzyme or clone has been reported [25–27]. In this paper, we report on the characterization of a Scots pine cDNA encoding a putative HMGS, the first HMGS DNA sequence described for plants. The influence of ozone, an abiotic stressor, on HMGS transcript levels and the possible importance of isoprenoids in plant defense reactions are discussed.

Scots pine seedlings were grown in Perlite and placed in a growth chamber at 75% relative humidity with a photoperiod of 14 h light/10 h dark and

```
   1  TGA TTG CAA AAG AGT GGG TTT TGT GCC TGG TAT TAT TAG CTG CTT TCC    48

  49  CAC TCT CAG GAT TTC TGA ATT TTC ATT CAG TCT GTC AAA ACA ATG GCA    96
   1                                                           M   A     2

  97  TCT CGT CCA GAG AAT GTT GGG ATT TTG GCT ATG GAG ATC TAC TTT CCT    14
   3   S   R   P   E   N   V   G   I   L   A   M   E   I   Y   F   P    18

 145  ACT ACA TGT GTC CAG CAG GAG GAC TTG GAA ACT TTT GAT GGA GTA AGT   192
  19   T   T   C   V   Q   Q   E   D   L   E   T   F   D   G   V   S    34

 193  AAA GGA AAA TAT ACA ATT GGT CTT GGA CAA GAC TGC ATG ACT TTC TGC   240
  35   K   G   K   Y   T   I   G   L   G   Q   D   C   M   T   F   C    50

 241  ACT GAC TTG GAA GAC GTT ATT TCT ATG AGT TTG ACA GCA GTA ACG TCA   288
  51   T   D   L   E   D   V   I   S   M   S   L   T   A   V   T   S    66

 289  CTT TTG GAA AAA TAT GAA ATT GAT CCA AAG CAA ATT GGT CGT TTG GAA   336
  67   L   L   E   K   Y   E   I   D   P   K   Q   I   G   R   L   E    82

 337  GTT GGC AGC GAA ACT GTT ATT GAC AAG AGC AAG TCA ATA AAG ACG TGG   384
  83   V   G   S   E   T   V   I   D   K   S   K   S   I   K   T   W    98

 385  TTG ATG CAT ATT TTT GAG AAG TGT GGT AAT ACT GAA ATT GAA GGT GTG   432
  99   L   M   H   I   F   E   K   C   G   N   T   E   I   E   G   V   114

 433  GAC TCA ACA AAT GCA TGC TAT GGA GGA ACT GCA GCC CTG TTT AAC TGT   480
 115   D   S   T   N   A   C   Y   G   G   T   A   A   L   F   N   C   130

 481  ATC AAC TGG ATT GAA AGC AGT TCT TGG GAT GGA AGA TAT GGT CTT GTT   528
 131   I   N   W   I   E   S   S   S   W   D   G   R   Y   G   L   V   146

 529  GTA GCT ACA GAC AGT GCA GTC TAT GCT GAA GGG GCA GCA CGA CCT ACT   576
 147   V   A   T   D   S   A   V   Y   A   E   G   A   A   R   P   T   162

 577  GGA GGA GCA GCT GCT GTT GCA ATG CTG ATA GGG CCT AAT GCT CCA ATA   624
 163   G   G   A   A   A   V   A   M   L   I   G   P   N   A   P   I   178

 625  GCA ACT GAG AGC AAA TAC AGG GGA ACA CAT ATG GCT CAT GTC TAT GAC   672
 179   A   T   E   S   K   Y   R   G   T   H   M   A   H   V   Y   D   194

 673  TTT TAT AAG CCT AAT CTG GCT AGT GAA TAT CCG GTT GTA GAT GGG AAG   720
 195   F   Y   K   P   N   L   A   S   E   Y   P   V   V   D   G   K   210

 721  CTT TCA CAA ACT TGT TAT CTC ATG GCA TTG GAT TCA TGC TAC AAA CGG   768
 211   L   S   Q   T   C   Y   L   M   A   L   D   S   C   Y   K   R   226

 769  TTT TGT AAT AAG TTT GAA AAG GAA GAA GGA AGA CAG TTT TCT CTT CTA   816
 227   F   C   N   K   F   E   K   E   E   G   R   Q   F   S   L   L   242

 817  GAT ACA GAT TAT ATA GCA TTT CAT TCT CCA TAC AAT AAG CTT GTA CAA   864
 243   D   T   D   Y   I   A   F   H   S   P   Y   N   K   L   V   Q   258

 865  AAG AGC TTT GGT CGC CTG TTG TTC AAT GAT TTC TCA AGA CAT GCC AGG   912
 259   K   S   F   G   R   L   L   F   N   D   F   S   R   H   A   R   274

 913  TCT GTT GGA AAG GAT GCA CAA GAG AAG CTA GAA CCC TTT GCT GGC TTG   960
 275   S   V   G   K   D   A   Q   E   K   L   E   P   F   A   G   L   290

 961  TCT GAA CAA GAT AGC TAC AAT AGT CGT GAC CTA GAA AAG GTT TCT CAG  1008
 291   S   E   Q   D   S   Y   N   S   R   D   L   E   K   V   S   Q   306

1009  CAG CTT GCG AAG CCA TTG TAT GAT GCA AAA ATT CAA CCA TCA ACT TTA  1056
 307   Q   L   A   K   P   L   Y   D   A   K   I   Q   P   S   T   L   322

1057  CTG CCA AAG CAA GTA GGC AAC ATG TAT ACA GCA TCT CTC TAT GCA GCA  1104
 323   L   P   K   Q   V   G   N   M   Y   T   A   S   L   Y   A   A   338

1105  TTA GCC TCC ATT ATT CAC AAC AAA CAT ACG ACT CTG GAT GGT CAA CGG  1152
 339   L   A   S   I   I   H   N   K   H   T   T   L   D   G   Q   R   354

1153  GTA ATG ATG TTC TCT TAT GGC AGT GGG CTT GCT TCT ACG TTG TTT TCT  1200
 355   V   M   M   F   S   Y   G   S   G   L   A   S   T   L   F   S   370

1201  TTT AAA ATC CGG GAG GGT CAA TTC CCT TTT ACT CTG TCA AAT ATT ACA  1248
 371   F   K   I   R   E   G   Q   F   P   F   T   L   S   N   I   T   386

1249  GAA GTT ATG GAC GTA CAA AAC AAA CTT GAT TCT CGT CAT GAG TTT CTT  1296
 387   E   V   M   D   V   Q   N   K   L   D   S   R   H   E   F   L   402

1297  CCT GAA GAT TTC GTG GAA AAC TTG AAG CGG ATG GAA ACT CTG TAC GGA  1344
 403   P   E   D   F   V   E   N   L   K   R   M   E   T   L   Y   G   418

1345  GCA AAG GAC TTC GTT TCA ACT TCT CAG CTC AGT TTG CTG CGG CCT GGC  1392
 419   A   K   D   F   V   S   T   S   Q   L   S   L   L   R   P   G   434

1393  GCT TTC TAT TTG ACT AAA GTA GAT TCC ATG TAC CGG CGT TTC TAT TCC  1440
 435   A   F   Y   L   T   K   V   D   S   M   Y   R   R   F   Y   S   450

1441  CGC AAA GTT ATT AGT GCT GGT GAC AAT TTT GAG AAG TCA AAA CTT GCC  1488
 451   R   K   V   I   S   A   G   D   N   F   E   K   S   K   L   A   466

1489  AAT GGT ACC ACC CAT GAT GAA TTG TAA ACA TGG TTT ATG TTT GTG AGG  1536
 467   N   G   T   T   H   D   E   L   *                               474

1537  TTA ACT AGT ACT GAT ATT CGT GCT GAA TGT AAG TTC TGC ATT GTA ACA  1584

1585  TAG CAT TCT GGA GTC TCC CAT TCC TTT TCT TTG GTA TCT GTT TAC TTT  1632

1633  TGT TTT AAG TAA ACG TGG TAG TGT TGA GAA AAT AAT TCA AAG GAT GCT  1680

1681  CCT TAA AGA GCA TTT GTT TGA ATC TCC TTT AGC CTC GGG GAT CTA TTT  1728

1729  TAA TTA CAA ATG AGC AGT GGG AAT AAA TTA AAT CAC TTC ATT GCA CTA  1776

1777  GGA AAC TTA GCT TGT TTG TGA CTT GGG ATA TAT TCA TCA GAA TAT AAC  1824

1825  ATG GGC GAT TTG CCA AGT TAA AAC GTG TAC TTC TCA TTT CAT CAG AAC  1872

1873  TTT CTT TGT GAT AGA TTT TGC TCA TCA AAG ATG TTG CAT TCT CAG CTG  1920

1921  GGC AGA GTG TGC GCT TTT ATT TAA GCT CAT GTT TGG GCA CTT ATT TG   1967
```

Fig. 1. Nucleotide and deduced amino-acid sequence of the Scots pine cDNA clone $O_3$I-3.5. The initiation consensus nucleotide sequence [34,35] and putative polyadenylation signals [36,37] are underlined. The stop codon is marked by an asterisk. The putative active site of clone $O_3$I-3.5 is marked in bold and underlined [23,42].

21/17°C day/night temperature [28]. Six-week-old seedlings were subjected to ozone (0.3 μl/l, 8 h) in acrylic cuvettes and ozone was generated by electrical discharge of dry oxygen [29]. 5 μg poly(A)$^+$ RNA from Scots pine seedlings treated with ozone for 4 h and 8 h, and an additional cultivation in clean air for 4 h, respectively, were used for cDNA cloning using the Superscript™ Plasmid System (Gibco BRL). Poly(A)$^+$ RNA was isolated as described elsewhere [13] and cDNA synthesis was according to the manufacturer's instructions. The cDNA was ligated into the plasmid pSPORT 1 (Gibco BRL) and transformation of *E. coli* JM 109 was as described elsewhere [30]. About $6 \times 10^5$ clones were differentially screened using the colony hybridization technique [31]. Hybridization of identical replica filters (about $2 \times 10^4$ clones/filter) was carried out with $^{32}$P-labeled poly(A)$^+$ RNA, isolated from ozone-treated or control seedlings, respectively. Clones of interest were subjected to a secondary and finally tertiary screening, resulting in 17 ozone-induced clones. Plasmid DNA of clone $O_3$I-3.5 was sequenced on both strands by primer hopping, using Ampli*Taq*® and *Taq*DyeDeoxy™ Terminator Cycle Sequencing Kits and an automatic fluorescence sequencer (ABI DNA Sequencer 373A). Primers were synthesized with a DNA synthesizer (Applied Biosystems 394A). DNA sequences were analyzed using software applications

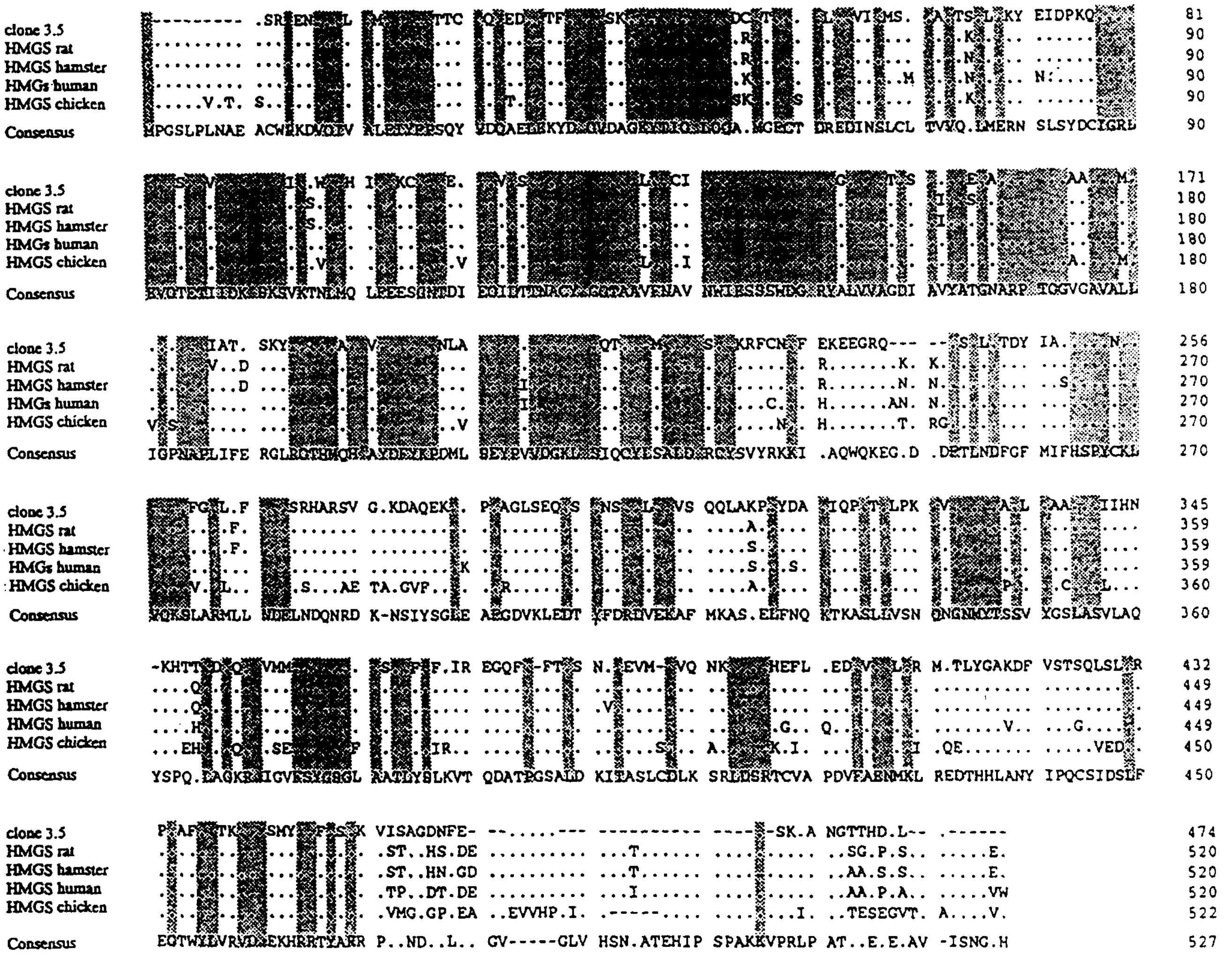

Fig. 2. Alignment of the predicted amino-acid sequence of clone $O_3$I-3.5 with human [43], rat [59], chinese hamster [24] and chicken [52] cytosolic HMGS.

of IntelliGenetics Suite and Wisconsin Package. Total RNA was isolated [32] and used for electrophoretic separation and transfer to nylon membranes. The blots were probed and washed as described [13]. RNA ladder 0.24–9.5 kb (Gibco BRL) was used as size marker. Radiolabeling of cDNA probes was done with the HexaPrimer Kit I (Appligene).

DNA sequence analysis of clone O$_3$I-3.5 showed an open reading frame of 1422 bp, 90 bp of a 5′ untranslated sequence, and a 3′ untranslated region of 455 bp. The DNA sequence and its encoded polypeptide is shown in Fig. 1. The coding sequence seems to be complete since there is an in-frame upstream TGA stop codon at position 64 before the ATG translation start site [33]. Furthermore, the initiation consensus sequence AACAATGGC found in plants [34,35], is present in clone O$_3$I-3.5 (underlined in Fig. 1). Nucleotide sequences following the stop codon showed the conserved A at position +1 and +3 [36]. The length of the cDNA insert without the poly(A) tail of 1967 bp corresponds well to the estimated size of a 1.95 kb transcript, detected by Northern analysis (Fig. 3). Taken together all these data indicate, that clone O$_3$I-3.5 is a full-length cDNA clone. The 5′ untranslated region consists of at least 90 bp, which is in the range of plant leader sequences [34]. In addition, the percentage of A + T with 62% fits to most leader sequences in plant genes [34]. The 3′ untranslated region contains two putative polyadenylation signals that are underlined in Fig. 1 [36,37]. The distance between the polyadenylation signals and the start of the poly(A) tail comprises an unusually high number of 212 or 299 base pairs, respectively. In most genes analyzed so far this signal sequence is about 10 to 33 bases in front of the polyadenylation site [36,38,39]. However, in petunia and maize up to 130 bp were found [37]. In plants not all AATAAA-like sequences can be recognized as a poly(A) signal [reviewed in [40]]. So it might be, that the 3′ untranslated region of clone O$_3$I-3.5 contains additional poly(A) signals [40].

Nucleic acid sequence comparisons of clone O$_3$I-3.5 using the EMBL and GenBank database showed no significant homologies to known DNA sequences. However, comparison of the deduced polypeptide of the open reading frame 1 with the SwissProt and PIR database showed a high similarity to cytosolic HMGS of vertebrates. In addition, the predicted molecular weight of 52 995 Da and the isoelectric point of 5.74 fit well to cytoplasmic liver HMGS [23,41]. Amino acids 105 to 125 (Fig. 1, marked in bold) showed a 16 out of 21 match with the active region of avian mitochondrial and hamster cytoplasmic HMGS (16 identical and 5 conserved amino acids) [23,42]. A sequence alignment of the predicted O$_3$I-3.5 amino acid sequence with known cytosolic HMGS sequences, using the SwissProt and PIR databases is shown in Fig. 2. A comparison of all five protein sequences showed 42% sequence identity and 56% similarity. A direct comparison of the amino acid sequence of clone O$_3$I-3.5 with human cytosolic HMGS [43] using the dotblot program according to [44] confirmed this homology (data not shown). In addition, the hydropathy profile [45] of the protein sequence of clone O$_3$I-3.5 was very similar to the profiles of known HMGS sequences (data not shown). Based on the primary amino acid sequence and the absence of an organelle transit peptide sequence, analyzed by the TRANSPEP program of PC/GENE®, clone O$_3$I-3.5 is localized in the cytosol. Clone O$_3$I-

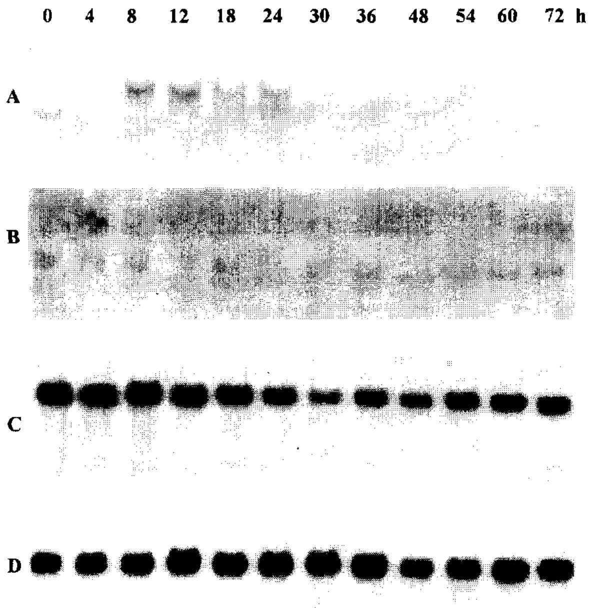

Fig. 3. Northern blot hybridization of clone O$_3$I-3.5 (A, B). Total RNA was isolated from ozone-treated (0.3 $\mu$l/l, 8 h) (A) and unfumigated (B) needles of Scots pine. Numbers correspond to the time period in h. As a control the membranes were rehybridized with a cDNA clone encoding the chlorophyll *a*/*b* binding protein of Scots pine (C, D); (C) + ozone, (D) − ozone.

3.5 has a HDEL motif at its C-terminus, known to be an endoplasmic reticulum (ER) targeting sequence [46]. In radish HMGS seems to be membrane-associated [26]. HMGR of *Arabidopsis* is thought to be localized in the ER [47,48]. Therefore the presence of this putative HMGS in the cytosol/ER boundary is in accordance with the hypothetical model of IPP synthesis in the cytosol (reviewed in [49,50]).

Expression of clone $O_3$I-3.5 was analyzed in ozone-treated Scots pine seedlings. In non ozone-treated needles of Scots pine seedlings clone $O_3$I-3.5 hybridized faintly to a 1.2 and 1.95 kb mRNA species (Fig. 3). Upon ozone treatment the 1.2 kb transcript decreased transiently after 4 h, and was again visible after further cultivation in clean air (Fig. 3). In contrast to this 1.2 kb mRNA species a 1.95 kb transcript increased transiently with a maximum after 8–12 h (Fig. 3). These results indicate the existence of two putative HMGS mRNA species in Scots pine, similar as described for mammals [51,52]. Quantitation of these changed mRNA amounts by densitometer scanning resulted in a decrease of the 1.2 kb transcript by a factor of 2, whereas the 1.95 kb transcript increased by a factor of 5. To demonstrate that no non-specific shift in the relative proportions of ribosomal RNA and mRNA occurred, we reprobed the membrane with a cDNA clone encoding the chlorophyll *a/b* binding protein (cab) from Scots pine (pINEab 13) [53]. Transcript levels of cab mRNA were transiently reduced by treatment with ozone (Fig. 3). This is in accordance with an ozone-induced cab transcript decrease in herbaceous plants [54]. As mentioned above, the mRNA size of 1.95 kb closely agrees to the full-length cDNA clone $O_3$I-3.5, corroborating that this mRNA species represents the clone. The different behaviour of the two HMGS transcripts indicates a complex response of the isoprenoid biosynthetic pathway to ozone, an abiotic stressor. Ozone treatment of plants results in an accumulation of cytosolic defense-related mRNA species (reviewed in [6,7]), whereas several transcripts of nuclear-encoded chloroplastic proteins are decreased [54–56]. Therefore it is tempting to speculate, that the 1.95 kb transcript corresponds to the cytosolic/ER HMGS, whereas the 1.2 kb mRNA species represents a chloroplastic protein. The isoprenoid biosynthetic pathway in plants is affected at the molecular level by several stress factors. Most studies have focused on HMGR levels, which respond to external stimuli like wounding, pathogen attack, elicitation, plant growth regulators and exogenous sterols (reviewed in [19,57]). However, in mammals a co-ordinated response of HMGS and HMGR activities was found [23,24]. Thus it seems, that HMGS may also be important for the regulation of the isoprenoid pathway in plants.

Our previous studies on ozone-induced transcript accumulations indicated a close similarity with transcript accumulations induced by pathogens or elicitors [2,3,11,58]. As isoprenoids are also involved in the plant defense against pathogens (reviewed in [57]), these results support the idea, that the expression of genes induced by pathogens or by ozone may be influenced via a similar signal transduction chain.

## Acknowledgements

The authors thank U. Linzner and G. Alschner for the primer synthesis. The plasmid pINEab 13 was kindly provided by Dr. S. Jansson (University of Umeå). This work was in part supported by EU-ROSILVA, 'Fonds der Chemischen Industrie' and Limagrain. The valuable suggestions made by an anonymous reviewer are gratefully acknowledged.

## References

[1] Treshow, M. and Anderson, F.K. (1989) Plant Stress from Air Pollution, Wiley, New York.
[2] Ernst, D., Schraudner, M., Langebartels, C. and Sandermann, H. (1992) Plant Mol. Biol. 20, 673–682.
[3] Eckey-Kaltenbach, H., Ernst, D., Heller, W. and Sandermann, H. (1994) Plant Physiol. 104, 67–74.
[4] Sharma, Y.K. and Davis, K.R. (1994) Plant Physiol. 105, 1089–1096.
[5] Willekens, H., Van Camp, W., Van Montagu, M., Inzé, D., Langebartels, C. and Sandermann, H. (1994) Plant Physiol. 106, 1007–1014.
[6] Kangasjärvi, J., Talvinen, J., Utriainen, M. and Karjalainen, R. (1994) Plant Cell Environ. 17, 783–794.
[7] Sandermann, H. (1996) Annu. Rev. Phytopathol. 34, 347–366.
[8] Hahlbrock, K. and Scheel, D. (1989) Annu. Rev. Plant Physiol. Plant Mol. Biol. 40, 347–369.
[9] Dixon, R.A. and Lamb, C.J. (1990) Annu. Rev. Plant Physiol. Plant Mol. Biol. 41, 339–367.
[10] Brederode, F.Th., Linthorst, H.J.M. and Bol, J.F. (1991) Plant Mol. Biol. 17, 1117–1126.

[11] Galliano, H., Cabané, M., Eckerskorn, C., Lottspeich, F., Sandermann, H. and Ernst, D. (1993) Plant Mol. Biol. 23, 145–156.

[12] Lewis, N.G. and Yamamoto, E. (1990) Annu. Rev. Plant Physiol. Plant Mol. Biol. 41, 455–496.

[13] Großkopf, E., Wegener-Strake, A., Sandermann, H. and Ernst, D. (1994) Can. J. For. Res. 24, 2030–2033.

[14] Schneiderbauer, A., Back, E., Sandermann, H. and Ernst, D. (1995) New Phytol. 130, 225–230.

[15] Kindl, H. (1991) Biochemie der Pflanzen, Springer, Berlin.

[16] Rudzicka, L., Eschenmoser, H. and Heusser, H. (1953) Experientia 9, 357–367.

[17] Stermer, B.A. and Bostock, R.M. (1987) Plant Physiol. 84, 404–408.

[18] Gondet, L. Weber, P., Maillot-Vernier, P., Benveniste, P. and Bach, T.J. (1992) Biochem. Biophys. Res. Commun. 186, 888–893.

[19] McGarvey, D.J. and Croteau, R. (1995) Plant Cell 7, 1015–1026.

[20] Yang, Z., Park, H., Lacy, G.H. and Cramer, C.L. (1991) Plant Cell 3, 397–405.

[21] Choi, D., Bostock, R.M., Avdiushko, S. and Hildebrand, D.F. (1994) Proc. Natl. Acad. Sci. USA 91, 2329–2333.

[22] Goldstein, J.L. and Brown, M.S. (1990) Nature 343, 425–430.

[23] Gil, G., Goldstein, J.L., Slaughter, C.A. and Brown, M.S. (1986) J. Biol. Chem. 261, 3710–3716.

[24] Mehrabian, M., Callaway, K.A., Clarke, C.F., Tanaka, R.D., Greenspan, M., Lusis, A.J., Sparkes, R.S., Mohandas, T., Edmond, J., Fogelman, A.M. and Edwards, P.E. (1986) J. Biol. Chem. 261, 16249–16255.

[25] Alam, A., Britton, G., Powls, R. and Goad, J. (1991) Biochem. Soc. Trans. 19, 164S.

[26] Weber, T. and Bach, T.J. (1994) Biochim. Biophys. Acta 1211, 85–96.

[27] Suvachittanont, W. and Wititsuwannakul, R. (1995) Phytochemistry 40, 757–761.

[28] Rosemann, D., Heller, W. and Sandermann, H. (1991) Plant Physiol. 97, 1280–1286.

[29] Langebartels, C., Kerner, K., Leonardi, S., Schraudner, M., Trost, M., Heller, W. and Sandermann, H. (1991) Plant Physiol. 95, 882–889.

[30] Inoue, H., Nojima, H. and Okayama, H. (1990) Gene 96, 23–28.

[31] Grunstein, M. and Hogness, D.S. (1975) Proc. Natl. Acad. Sci. USA 72, 3961–3965.

[32] Chang, S., Puryear, J. and Cairney, J. (1993) Plant Mol. Biol. Rep. 11, 113–116.

[33] Kozak, M. (1984) Nucleic Acids Res. 12, 857–872.

[34] Joshi, C.P. (1987) Nucleic Acids Res. 15, 6643–6653.

[35] Lütcke, H.A., Chow, K.C., Mickel, F.S., Moos, K.A., Kern, H.F. and Scheele, G.A. (1987) EMBO J. 6, 43–48.

[36] Joshi, C.P. (1987) Nucleic Acids Res. 15, 9627–9640.

[37] Dean, C., Tamaki, S., Dunsmuir, P., Favreau, M., Katayama, C., Dooner, H. and Bedbrook, J. (1986) Nucleic Acids Res. 14, 2229–2240.

[38] Wahle, E. and Keller, W. (1992) Annu. Rev. Biochem. 61, 419–440.

[39] Proudfoot, N.J. and Brownlee, G.G. (1976) Nature 263, 211–214.

[40] Wu, L., Ueda, T. and Messing, J. (1995) Plant J. 8, 323–329.

[41] Clinkenbeard, K.D., Sugiyama, T., Reed, D. and Lane, M.D. (1975) J. Biol. Chem. 250, 3124–3135.

[42] Miziorka, H.M. and Behnke, C.E. (1985) J. Biol. Chem. 260, 13513–13516.

[43] Russ, A.P., Ruzicka, V., Maerz, W., Appelhans, H. and Groß, W. (1992) Biochim. Biophys. Acta 1132, 329–331.

[44] Maizel, J.V. and Lenk, R.P. (1981) Proc. Natl. Acad. Sci. USA 78, 7665–7669.

[45] Kyte, J. and Doolittle, R.F. (1982) J. Mol. Biol. 157, 105–132.

[46] Bairoch, A. (1993) Nucleic Acids Res. 21, 3097–3103.

[47] Enjuto, M., Balcells, L., Campos, N., Caelles, C., Arró, M. and Boronat, A. (1994) Proc. Natl. Acad. Sci. USA 91, 927–931.

[48] Lumbrears, V., Campos, N. and Boronat, A. (1995) Plant J. 8, 541–549.

[49] Kleinig, H. (1989) Annu. Rev. Plant Physiol. Plant Mol. Biol. 40, 39–59.

[50] Chapell, J. (1995) Plant Physiol. 107, 1–6.

[51] Ayté, J., Gil-Gómez, G., Haro, D., Marrero, P.F. and Hegardt, F.G. (1990) Proc. Natl. Acad. Sci. USA 87, 3874–3878.

[52] Kattar-Cooley, P.A., Wang, H.-H.L., Mende-Mueller, L.M. and Miziorko, H.M. (1990) Arch. Biochem. Biophys. 283, 523–529.

[53] Jansson, S. and Gustafsson, P. (1990) Plant Mol. Biol. 14, 287–296.

[54] Bahl, A. and Kahl, G. (1995) Environ. Pollut. 88, 57–65.

[55] Reddy, G.N., Arteca, R.N., Dai, Y.-R., Flores, H.E., Negm., F.B. and Pell, E.J. (1993) Plant Cell Environ. 16, 819–826.

[56] Conklin, P.L. and Last, R.L. (1995) Plant Physiol. 109, 203–212.

[57] Weissenborn, D.L., Denbow, C.J., Laine, M., Lang, S.S., Yang, Z., Yu, X. and Cramer, C.L. (1995) Physiol. Plant. 93, 393–400.

[58] Ernst, D., Bodemann, A., Schmelzer, E., Langebartels, C. and Sandermann, H. (1996) J. Plant Physiol. 148, 215–221.

[59] Ayté, J., Gil-Gómez, G. and Hegardt, F.G. (1990) Nucleic Acids Res. 18, 3642.