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3-Hydroxy-3-methylglutaryl-CoA reductase gene of *Gibberella fujikuroi*: isolation and characterization

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Abstract 3-Hydroxy-3-methylglutaryl-CoA reductase (HMGR) is the first specific enzyme of the isoprenoid pathway, which leads to several classes of primary and secondary metabolites such as sterols, quinones, carotenoids and gibberellins. The structural gene of HMG-CoA reductase was isolated from the ascomycetous fungus Gibberella fujikuroi. Additionally, the most conserved region of this gene was also isolated from another plant pathogenic fungus, Sphaceloma manihoticola. Both ascomycetous fungi use the plant hormone gibberellin to induce an elongation of infected host plants, and in the case of S. manihoticola of plant tumors. Sequence analysis revealed a high degree of similarity between the deduced amino-acid sequences in the C-terminal catalytic domains of all known HMG-CoA reductases, but the highest degree was found between the sequences of both analysed ascomycetes. In contrast to Saccharomyces cerevisiae, Ustilago maydis and plants, G. fujikuroi and S. manihoticola possess only a single copy of this gene, although the product of HMGR (mevalonate) is the precursor for essential sterol and quinone biosynthesis and secondary metabolites such as gibberellins. RNAblot and hybridization experiments showed that gene expression is not influenced by either glucose or ammonium excess.

Key words 3-hydroxy-3-methylglutaryl-CoA reductase · Isoprenoids · Gibberellins · *Gibberella · Sphaceloma*

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Introduction

Gibberella fujikuroi is a rice pathogenic fungus producing high levels of gibberellins, a family of diterpenoid plant hormones. The identification of gibberellin as a plant growth regulator is an example of the interaction between a pathogen and its host plant. When the fungus attacks rice seedlings, an early disease symptom is the super-elongation of the shoots ("bakanae" disease). In 1935 Yabuta announced the isolation of a growth stimulant from the culture fluid of the fungus. The substance was called "gibberellin" according to the bakanae fungus. Only in 1956, however, gibberellins were found in extracts of higher plants (Radley 1956) and then defined as natural plant hormones, controlling such diverse processes as seed germination, cell elongation and division, flowering and fruit development. Extensive biochemical studies on endogenous gibberellin (GA) intermediates in GA-responsive plant dwarf mutants and GA-deficient mutants of G. fujikuroi have allowed the determination of the GA biosynthetic pathway (Bearder 1983; Graebe 1987). As diterpenes, the gibberellins are formed in the isoprenoid pathway, starting from mevalonic acid which is converted via isopentenyl, dimethylallyl, geranyl and farnesyl pyrophosphates to geranylgeranylpyrophosphate, being an important shared intermediate. Mevalonic acid is produced by the key enyzme 3-hydroxy-3-methylglutaryl-CoA reductase (EC 1.1.1.34) from 3-hydroxy-3-methylglutaryl-CoA.

The 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) gene has been isolated from several organisms such as *Saccharomyces cervisiae* (Basson et al. 1988), *Mesocricetus aureatus* (Chin et al. 1984; Skalnik and Simoni 1985), *Drosophila melanogaster* (Gertler et al. 1988), *Arabidopsis thaliana* (Learned and Fink 1989), *Hevea brasiliensis* (Chye et al. 1991 a, b) and *Ustilago maydis* (Croxen et al. 1994) because of its importance for sterols, and secondary metabolite biosynthesis. Whereas in animals only one HMGR gene copy was found, two ore more HMGR gene copies could be isolated from plants. For *H. brasiliensis* it was demonstrated that different HMGR

genes are differently regulated by ethylene (Chye et al. 1991 b). In the plant pathogenic basidiomycete *U. maydis* two HMGR isoenzymes were found (Croxen et al. 1994).

In mammals the synthesis and activity of this rate-limiting enzyme is regulated by negative feed back on steady state levels of HMGR mRNA as well as on the level of enzyme activity. For regulation at both levels, two products of the terpenoid pathway are necessary: a sterol (the so called low-density lipoprotein) and, second, a non-sterolic product which so far has not be determined exactly. Regulation of HMGR activity seems to be different in animals and plants. All structural genes of HMGR which have been analyzed to-date are highly conserved with respect to their structure and function. The enzymes concerned all possess two domains: the NH₂-terminal region encompasses the membrane-bound region whereas the COOH-terminal region of the protein is located in the cytoplasm and contains the biologically active domain. The N-terminal membrane anchor and the C-terminal catalytic domain are separated by a non-homologous linker region.

In mammals experimental evidence suggests that membrane attachment of HMGR is a prerequisite for the regulation of enzyme activity (Gil et al. 1985; Luskey and Stevens 1985). It is not known if such a regulation mode exists also in plants, in which the membrane-bound region is reduced from seven to two transmembrane domains.

Seven transmembrane domains are also assumed in yeast HMG-CoA reductases though it still has to be determined if HMG-CoA reductases have the same structure in filamentous fungi and if the structural similarity to mammalian proteins results in a similar regulation.

In the present study, we report the isolation and analysis of the complete genomic sequence of HMGR gene of the *G. fujikuroi* wild-type strain m567. The deduced aminoacid sequence was compared to HMGR sequences from other organisms. Furthermore, the most conserved region of the gene was isolated from the cassava pathogen *Sphaceloma manihoticola* which is well known as a producer of gibberellin GA_4 . The HMGR gene copy number was estimated for both fungi. RNA-blot experiments showed that the HMGR gene is not the target for C-and N-catabolite repression of gibberellin biosynthesis.

Materials and methods

Strains and culture conditions. G. fujikuroi m567 is a wild-type rice isolate which was provided by the Culture Collection for Fungi, Weimar (Germany). Strain G. fujikuroi MBC31 is a methyl-benzimidazol-2-yl carbamate-treated derivative of m567 with a reduced DNA content and a reduced GA-production level (B. Tudzynski, unpublished).Wild-type strain S. manihoticola Lu949, which was isolated from Cassava, was provided by W. Rademacher, BASF. Stock cultures of the strains were maintained on potato-saccharose agar (10% potatoes, 2% saccharose, 0.7% CaCO₃, 2% agar).

RNA-blot and hybridization. Cultivation for transcription analysis under inducing conditions was carried out in a GA₃ production medium [6% plant oil, 0.05% (NH₄)₂SO₄, 1.5% corn-steep liquor, 0.1% KH₂PO₄, pH 5.5]. For cultivation under GA-repressing conditions

10% glucose, 0.05% (NH₄)₂SO₄, 1.5% corn-steep liquor (Sigma), 0.1% KH₂PO₄, pH 5.5, was used. For the analysis of transcription level under N-excess or limitation and C-excess or limitation, a minimal medium with 1% ammonium, or without ammonium, and 10% glucose, or without glucose, respectively, was used. RNA was isolated by the urea / LiCl procedure (Chambers and Russo 1986). Total RNA was size-fractionated by electrophoresis on formaldehyde agarose gels (Church and Gilbert 1984) and downward blotted onto Hybond-N filters (Amersham, UK) (Sambrook et al. 1989). Pre-hybridization and hybridization were carried out at 60°C in 1% BSA fraction V, 1 mM EDTA, 7% SDS, 0.25 M NaHPO₄, pH 7.2. For hybridization a radioactively labeled fragment of the G. fujikuroi HMG-CoA reductase gene was used as the probe. Filters were washed twice with washing buffer 1 (0.5% BSA fraction V, 1 mM EDTA, 5% SDS, 40 mM NaHPO₄, pH 7.2) at room temperature, once with washing buffer 1 at 60°C for 15 min, and finally twice with washing buffer 2 (1 mM EDTA, 1% SDS, 40 mM NaHPO₄, pH 7.2) for 2 min at room temperature. The gpd gene of Claviceps purpurea was used as a control for RNA transfer (Jungehülsing et al. 1994).

Library screening. Plaques from a G. fujikuroi m567 genomic DNA library in phage EMBL3 were transferred onto nylon filters (Sambrook et al. 1989). A 123-bp PCR fragment derived from the genomic DNA of G. fujikuroi was used to probe the nylon membranes under conditions of high stringency (Sambrook et al. 1989). Phages yielding a positive signal on duplicate filters were purified, restriction-analysed, and probed with the PCR fragment. Hybridizing fragments were isolated and cloned into pUC vectors. Recombinant plasmids were propagated in E. coli DH5 α .

Preparation of G. fujikuroi genomic DNA and phage DNA. G. fujikuroi genomic DNA and the DNA of lambda phage were isolated according to the standard protocols of Cenis (1992) and Sambrook et al. (1989), respectively.

DNA-sequencing and sequence analysis. Fragments of genomic DNA clones, carrying parts of the HMGR-gene, were subcloned into pUC19. Sequencing with appropriate primers was performed for both DNA strains, using a Pharmacia T7-Sequencing Kit. The sequence data analysis, including the construction of trees, was performed with the program "Husar" (Heidelberg).

PCR. PCR conditions were: 94° C for 4 min, 30 cycles of 54° C for 1 min, 72° C for 1.5 min, 94° C for 1 min. Reactions contained 1×Super *Taq* reaction buffer (HT Biotechnology Ltd), 200 mM dNTPs, 50 ng of each primer, 50 ng template DNA, 0.5 units of Super *Taq* polymerase (HT Biotechnology Ltd). In order to isolate the HMG-CoA reductase gene from *G. fujikuroi*, two oligonucleotides were synthesized using the standard eukaryotic nuclear gene codon assignments (Gurr et al. 1987):

HMG 1 5'-GAT/C GCA/T ATG GGG/T ATG AAT/C ATG AT-3' 3'-CTA/G CGT/A TAC CCC/A TAC TTA/G TAC TA-5'

HMG 2 5'-AAU/C UGG AUU/C GAG GGN CG-3' 3'-TTA/G ACC TAA/G CTC CCN GC-5'

Both primers were used as mixtures of both strands. Primer sequences correspond to two conserved regions within the HMG-CoA reductase proteins derived from following organisms: *S. cerevisiae* (Basson et al. 1988), *A. thaliana* (Learned and Fink 1989), *M. aureatus* (Chin et al. 1984) and *D. melanogaster* (Gertler et al. 1988). Another set of oligonucleotides was used for the isolation of a terminal region of the *S. manihoticola* HMGR gene by PCR:

HMG 4 5'-AAU/C UGG AUU/C GAG GGN CG-3'

HMG 3 5'-ATG CTG GGC AAG ATC CCT GG-3'

The oligonucleotide primer HMG3 was synthesized on the basis of the sequence data of the *G. fujikuroi* HMGR gene. Primer HMG 4 was synthesized according to the alignment of the HMGRs of *S. cerevisiae*, *A. thaliana*, *M. auratus* and *D. melanogaster*.

Southern-blot analysis. Digestion of the genomic DNA of G. fujikuroi with restriction enzymes and gel electrophoresis were done as described by Sambrook et al. (1989). Electrophoresed digested DNA was transferred onto Hybond-N filters (Amersham, UK) using the vacuum blotting method and hybridized at high-stringency conditions (Sambrook et al. 1989). Hybridization probes were prepared by random oligonucleotide priming using a GIBCO random prime labeling kit and the GIBCO protocol. Blots were washed twice with $1 \times$ SSPE, 0.1% SDS at 65°C for 15 min each; the final wash was carried out in 0.1×SSPE, 0.1%SDS at 65°C for 30 min.

Results

Isolation of the *G. fujikuroi* HMG-CoA reductase (HMGR) gene

Two oligonucleotides were synthesized to isolate the HMGR gene from G. fujikuroi. These primers encode the following peptide sequences relating to two conserved regions within the HMGR proteins of different organisms (see Materials and methods): HMG 1, Asp Ala Met Gly Met Asn Met; HMG 2, Asn Trp Ile Glu Gly Arg. Amplification of a corresponding DNA fragment of the G. fujikuroi gene was accomplished by PCR using these primers with G. fujikuroi m567 genomic DNA as a template. A single amplified DNA product (123 bp) was obtained and employed as a homologous probe to identify genomic DNA clones from a G. fujikuroi m567 EMBL3 library. Approximately 40 000 recombinant clones were screened at high stringency. Two positive plaques, designated 118/9 and 124/1, were isolated and purified. The DNA obtained from both λ clones was digested with restriction endonucleases (EcoRI, SalI, BamHI, XhoI, HindIII, PstI, XbaI and combinations of *EcoRI/SalI* and *SalI/BamHI*) and analyzed by Southern hybridization using the PCR fragment as a probe. Restriction analysis revealed that both λ clones contain overlapping inserts. Five different strongly hybridizing fragments of these λ clones were chosen for cloning into pUC vectors (Table 1). The 7.5-kb EcoRI fragment derived from $\lambda 18/9$ (designated pJ25) contains the 1.05-kb Sall/ *Bam*HI, as well as the 4.2-kb *Eco*RI/*Sal*I, fragment of λ 24/1 (Fig. 1) and overlaps the 10.0-kb *PstI* and 5.0-kb *HindIII* fragments of plasmids pJ15 and pSTA1101 (Table 1) at the 5' and 3' ends, respectively. The smallest cloned 1.05-kb Sall/BamHI fragment from $\lambda 24/1$ codes for the main part of the catalytic domain of the HMGR of G. fujikuroi.

Fig. 1 Physical map of plasmid pJ25 containing the *G. fujikuroi* HMGR gene. The *light dotted box* shows the position of the coding region and the dark dotted box indicates the position of the putative intron

Table 1 List of fragments chosen for cloning into pUC vectors

No.	Plasmid	Insert size (kb)	Cloning vector	Original phage	Restriction fragment			
1	pSTA1100	1.05	pUC13	$\lambda 24/1$	SalI/BamHI			
2	pSTA1101	5.0	pUC13	$\lambda 18/9$	HindIII			
3	pSTA1102	4.2	pUC18	$\lambda 24/1$	Sall/EcoRI			
4	pJ15	10.0	pUC19	$\lambda 24/1$	PstI			
5	pJ25	7.5	pUC19	$\lambda 18/9$	<i>Eco</i> RI			

For DNA sequencing, relevant regions of the pJ25 insert were subcloned and sequenced on both strands using universal and reverse primers as well as specific oligonucleotides. The nucleotide and deduced amino-acid sequence of the HMGR gene are shown in Fig. 2. This region contains the structural gene and regulatory regions of the HMGR of G. fujikuroi. The sequence starts 769 bp upstream of a putative initiation codon (ATG) and continues 978 bp beyond a stop codon (TAA). This region of 2928 bp encodes a protein of 976 amino acids with a molecular mass of 105 449 Da. The ORF is interrupted in the strongly conserved domain b2 (Liscum et al. 1985) by a predicted 47-bp intron at position 2531. This deduced intron possesses typical fungal 5' and 3' splicing sites as well as the internal lariat consensus sequence. The intron position between G. fujikuroi and A. thaliana HMGR gene 1, H. brasiliensis HMGR gene1 and HMGR gene 3 and M. auratus HMGR gene is not conserved. The other fungal HMGRs which have been analyzed do not possess introns.

The nucleotide sequence surrounding the supposed translation initiation site (Fig. 2 marked with +1) is comparable to the Kozak consensus sequence which is described for translation initiation in fungi: the -3 position is an adenine (Gurr et al. 1987; Unkles 1992). In the promotor region no CAAT motif could be found; a TATAAA box is located at position -166 (TATTAC). Five putative CT motifs were present at positions -95, -153, -434, -517 and -716. Such CT-rich sequences are particularily obvious in genes lacking TATAAA and CAAT motifs and in highly expressed genes, suggesting their role as possible promotor elements (Gurr et al. 1987). Putative binding sites (GATA) for a nitrogen regulatory protein (AREA, NIT2) were found at positions -171, -189, -190, -496, -631 and -633 (AREA: Kudla et al. 1990; NIT2: Fu and Marzluf 1990). Gap alignment analysis of the deduced amino-acid sequence of the G. fujikuroi HMGR gene with the U. may-

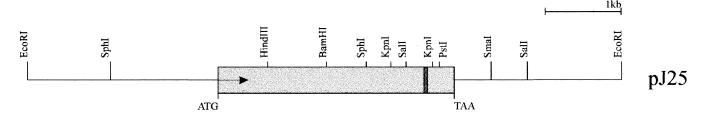


Fig. 2 Nucleotide sequence of the HMGR gene of G. fujikuroi and its deduced amino-acid sequence (accession number X94307). The nucleotides are numbered relative to the putative translation start site ATG. The putative intron is shown in lower-case letters, its intron consensus sequences are marked by underlined letters, the Kozak-consensus sequence for the translation start point is marked by dotted underlining, the translation start point itself is written in *bold letters*. Promotor motifs are marked, putative TATA box (bold letters), pyrimidine-rich tracks (double underlined), putative GATA motifs (under- and over-lined, respectively) (Fu and Marzluf 1988; Kudla et al. 1990), a putative binding site for a CREA like protein (in *italics*). The stop codon in *bold* is marked by three asterisks, the putative polyadenylation signal is underlined. Primer sequences which were used for S. manihoticola PCR have dotted underlines

GTCAATGGCTACTTCAAATACCCGCCTTTTCTCCAGA<u>TCTTTTCTTCTTCCCC</u>GGACTTACACCTGCGCTTGAGCCAAAATCAATGTGGTAAACGACATCGACGACGACATAGCCCTTCTAATC -649 CTCGAATCGTACCC<u>GATA</u>TCATGGCTGCGATTCTCCTGCCGCAGCGGTTCCGCGGCGAAGCTCCCGTCACCGAAAAAACTACGCCGTCATGGGCCTCCAAGCGACTTACGCCCATCGCCC G<u>TTCCTCTCCC</u>GACTCGCGTGCTCTCACCCGAATAACAACAACCGTCGTCGCCGTCGCCGTCCTTGCGAGGCACCTCATACGTCGG<u>CCTTCTCC</u>AGGAGAGCCTCTTCAACACCGATGT TGAAAGCGCTACTCTTGGCAAGGCCGATTGGTCTACCCTCGTGGAAGGCAGTCGAGTCCTACGCGCAGACCTGAAACTGCTTGGAACTGGAAGGCCGTCGAGCAGGATGTCGTTGCCGAT GCTGGCTCGGATGCCGACCACCTTGCCCTCAGGGACTCTTGTCTTCCAGCAGGCCTCTGCGGAGTCCTCCAGCACTGCTCTCACCACCACGTGC<u>CTAT</u><u>CCCCCAGA</u>ACCTGT<u>CTAT</u> TACSTCTCCCCTTACCGAGAATCCGTTCACCGCCTACTCTCAGGACAGCATACTCGCCTACGCCTACTCCCTAGGGGTCCCGAGTTCCTTGCTGCCCAAGAGATTCCCAA -49 +1. $\textbf{M} \hspace{0.5cm} \textbf{D} \hspace{0.5cm} \textbf{H} \hspace{0.5cm} \textbf{E} \hspace{0.5cm} \textbf{G} \hspace{0.5cm} \textbf{C} \hspace{0.5cm} \textbf{H} \hspace{0.5cm} \textbf{P} \hspace{0.5cm} \textbf{Q} \hspace{0.5cm} \textbf{C} \hspace{0.5cm} \textbf{C} \hspace{0.5cm} \textbf{W} \hspace{0.5cm} \textbf{V} \hspace{0.5cm} \textbf{S} \hspace{0.5cm} \textbf{N} \hspace{0.5cm} \textbf{A} \hspace{0.5cm} \textbf{W} \hspace{0.5cm} \textbf{S} \hspace{0.5cm} \textbf{E}$ 24 TTCCTCGACCTTCTCAAGAACGCCGAGACTCTCGATATCGTCATCATGCTTTCTGGTTACATTGCCATGCACTTGACATTTGTCTCCCCTCTTCATGCGTAAGATGGGTTCTAAG 192 FLDLLKNAETLDIVIMLLGYIAMHLTFVSLFLSMRKMGSK 64 TTCT6GCTC6GCATCT6TACTCT6TTCTCCTCTC6CCTTCCTCTCCGCCTCCGCCACCACGAA6CT6GGTGTTCCCATCACGCGTCATTCTTCT6TCT6AC6GGTCTCCCCTTC 312 FWLGICTLFSSVFAFLFGLVVTTKLGVPISVILLSEGL F 104 432 CTGGTIGTCACCATTGGCTTCGAGAAGAACATTGTTCTCCCCCCGCGTGTCATGTCCCCCCCTGTGAGCACCGACGAATCCAGGCTCAGAACTCCAAGTCTGGCAAGCGATCTCCCGAC L V V T I G F E K N I V L T R A V M S H A I E H R R I Q A Q N S K S G K R S P D 144 552 GGTTCCACGCAGAACATGATCCAGTATGCTGTTCAGGCCGCCATCAAGGAGAAGGGTTTCGAGATCATTCGCGACTACGCTATTGAGATTGTCATTCTTGTCATTGGTGCTGCTTCTGGT 184 G S T Q N M I Q Y A V Q A A I K E K G F E I I R D Y A I E I V I L V I G A A S G GTTCAGGGTGGTCTCCAGCAGTTCTGCTTCCTGGCCGCCTGGACCTTGTTCTTCGACTTCATCCTCTTCACATTCTACACTGCCATCCTCAGCATCAAGCTTAGATCAACCGTATCA 672 V Q G G L Q Q F C F L A A W T L F F D F I L L F T F Y T A I L S I K L R S T V S 224 AGGGTGATGTGGGTATGGGTGATGGCCCTTGAGGGTGATGGCGTCACGTCGTGTTGCCGAGAATGTCGCGGAGGGTGATGATGAGCGGGTGACGCCCTCTTATTTGGC 7.92 S V M S I C V W P L R M M A S R R V A E N V A K G D D E L N R V R G D A P L F G 264 CGCAAGAGCAGCAGCATTCCCAAGTTCAAGGTCTTGATGATTCTCGGCCTTCATCTTCGTCAACATTGTCAACATCTGCTCGATCCCATTCCGCAACCCTAGCTCTATGTCCACCATCCGA 912 R K S S S I P K F K V L M I L G F I F V N I V N I C S I P F R N P S S M S T I R 304 1032 TWASSLGGVIAPLSVDPFKVASNGLDAILPTAKSNNRPTL 344 GTCACTGTCTTGACTCCATCAAGTACGAGCTCGAGTACCCTTCGATCCACTACGCTCTTGGTTCTGCCGCCAGCAACCCTGCCTACAACGATGCTTTCCACCATCACTTCCAGGGCTAC 1152 TVLTPIKYELEYPSIHYALGSAASNPAYNDAFHHHFQG 384 GGTGTCGGCGGCCGCATGGTTGGCGGCATTCTCAAGTCTCTCGAGGATCCCGTCCTCCAAGTGGATTGTCATTGCCCTTGCTCTCAGCGTCGCTCTTAACGGCTACCTCTTCAACGTT G V G G R M V G G I L K S L E D P V L S K W I V I A L A L S V A L N G Y L F N V 424 GCCCCCTGGGGAATCAAGGACCCCAATGTTCCTGAGCACAACATCGACCGAAACGAACTTGCCCGTGCTCGCGAGTTTAACGACACCGGCTCCGCTACTCTCCTCGGCGAATACGTG 1392 A R W G I K D P N V P E H N I D R N E L A R A R E F N D T G S A T L P L G E Y V 464 CCTCCCACTCCCATGCGAACCCAACCCAGCACTCCTGCTATTACCGACGACGAGGGCCGAAGGTCTTCACATGACAAAGGCTCGACCTGCCCAACCGAGCAACGAAGAACTT 1512 P T P M R T Q P S T P A I T D D E A E G L H M T K A R P A N L P N R S N E E L 504 GAGAAGCTCTTGTCTGAGAACGCCCTGCGCGAGATGACCGACGAGGAGGTTATTTCTCTGTCTATGCGTGGCAAGATCCCTGGTTACGCACTTGAAAAGACCCTCGGTGACTTCACTCGC 1632 E K L L S E N A L R E M T D E E V I S L S M R G K I P G Y A L E K T L G D F T R 544 A V K I R R S I I A R N'K A A A D I T H S L D R S K L P Y E N Y N W E R F F G A

dis HMGR1 and the *S. cervisiae* HMGR1 and HMGR2 proteins revealed 64.3%, 64.1% and 65.8% overall similarity, repectively. This similarity extends from 76 up to 83% in the conserved C-terminal portion of HMGR gene product. The hydrophobicity plot of the *G. fujikuroi* protein revealed seven peaks of hydrophobicity within the N-terminal domain which may be transmembrane domains (data not shown). In *S. cerevisiae* HMGR1 and HMGR2, as well as in *U. maydis* HMGR1, eight hydrophobic regions have been identified, but only seven of them have been recognized as membrane-spanning domains (Sengstag et al. 1990).

HMGR gene copy number

Previous experiments revealed that the mononucleate microconidia of the wild-type strain *G. fujikuroi* m567 are difficult to mutagenize. On the basis of the DNA content of strain m567 and its benomyl-treated derivative *G. fujikuroi* MBC31, it could be demonstrated that *G. fujikuroi* MBC31 possesses 50% of the DNA content of the wild-type strain m567. Additionally, *G. fujikuroi* MBC31 is characterized by a reduced GA₃ yield (tenfold less than the GA production of m567) and a tenfold increased rate of mutant production after the same UV treatment as m567

Fig. 2 (continued)

TGCTGI				•			•			•			•						٠						٠							•			•
	GAG	ACG	TAT	TGG/	TACI	ATGC	CTCI	TTCC	CCG	ICGG	TGT	TGC	TGG	TCC	TCT:	FGTO	CATCO	GATG	GAC	AGAGC	TACI	TCP	TCC	CCA	TGG	CTP	CTA	CTO	GAGG	GTG	TCT	TGG	TTG	GCC.	AGT
СС	Е	Ν	V	I	; Y	М	Ρ	\mathbf{L}	Ρ	V	G	V	A	G	P I	L I	Ι	D	G	Q S	Y	F	Ι	Ρ	М	А	т	Т	Е	G	V	L	V	A	S
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SCCAGI	CGA	GTT	GCAA	GGCC	ATC!	AACT	CTG	GTGC	GTG	GTGC	CAT	CAC	TGT	TCT	CACI	rgc'i	GATO	GTA	TGA	CTCGI	GGTC	CTI	GTG	TCG	CTI	TCG	GAGA	CTC	TTG	AGC	GCG	CTG	GTO	GCT	GCC
A S	R	G	С	ΚJ	I	Ν	s	G	G	G	A	Ι	Т	v	L	r 7	D	G	М	T F	G	Ρ	С	V	A	F	Е	Т	L	Е	R	A	G	A	A
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AGCTC	TGG	CTTG	ACTC	TGAA	GCT	GGTC	AGGI	ATAT	rga'i	IGAA	GAA	GGC	TTT	CAA	CTC	AACO	AGTO	GCT	TCG	CCCGC	CTCC	CAGI	CCA	TGA	AGA	CCC	CTC	TTG	CTG	GTA	CCA	ACC	TGI	FAC.	ATT
ΚI	W	L	D	S I	C A	G	Q	D	М	М	К	К	A	F	N S	5 1	S	R	F	A F	L	Q	S	М	Κ	т	А	L	A	G	Т	Ν	\mathbf{L}	Y	I
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GATTC	AAG/	ACCA	CCAC	CGGI	GACO	ют <u>а</u>	TGG	<u>TA</u>	<u>rga</u>	ACAT	GAT	TTC	CAA	GGG.	AGTO	CGAC	CACO	CTC'	TAA	GCGTC	ATGO	GCCA	ACG	ATG	GAG	GTI	TCG	ACG	GACA	TGC.	AGA	TCP	TCI	ICT	GTC
R F	К	Т	Т	T	; D	А	М	G	М	N	М	Ι	S	K	G١	V E	H H	А	L	s V	M	А	Ν	D	G	G	F	D	D	М	Q	I	Ι	S	V
				·			·			·									•			·			·							•			·
CTGGC	AAC'	CACT	GTAC	GGAI	AAG	AAGG	CCGG	CGGC	CCCI	ICAA	CTG	GAT	CGA	CGG.	ACGI	rgg1	AAGG	GTG	TTG	TCGCI	GAGO	GCTA	TCA	TTC	CCG	GTG	GAGG	TCO	TCC	GCA	GCG	TTC	TCA	۹AG.	AGC
s e	N	Y	С	Τſ	K	К	А	А	A	L	N	W	I	D	G F	R (F K	G	v	V P	Е	А	I	I	Ρ	G	E	V	v	R	S	V	\mathbf{L}	К	\mathbf{S}
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SATGTC	GAC.	CTC	FTGT	TGAG	CTC	\ACG	TTGC	CTAF	AGA/	ACTT	GAT	TGG	TTC	TGC	TATO	GCI	GGTI	'CAG'	TTG	GTGGI	TTCF	ACG	iccc	ACG	CTG	CCA	ACA	TTG	TCG	CTG	CTA	TTT	TCC	CTG	SCC
D V	D	S	L	VE	L	Ν	V	А	Κ	Ν	L	I	G .	s.	A M	4 F	G	S	V	G	F	Ν	A	Н	A	A	Ν	Ι	V	A	А	Ι	F	L	А
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CTGGA	CAG	GACO	CTGC	TCAC	GTTO	GTCG	AGA	GCGC	CCAF	ATTG	TAT	CAC	CAT	CAT	GAA	GAAg	rtaag	tt	gtc	togca	cato	aag	tcg	gaa	aca	tgc	taa	cgt	cca	tag	CCT	CAP	TGG	GAG	CTC
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S E TGGAG AGACC TGCAC	TCAJ V TGAJ ACTI	A AAGC K TGG CGC.	? S GATA A I ICAC AATT ACTT	R GACC D ACAC CAA1 GTC/	S AA T E * KCATC	T NACT ** GGAG AATC	T E TTGC TTTT	PTGC	FTG CATT FGTC FTGT	AAGG IGAA JAAA	agt. Cat TCA	aga tct tgc	GGC GGA ATA GAC	GAG AAG. TGT	CGGG ATGA CTTC	AAAC	CATC CATC	<u>AA</u> G :C <u>AT</u> :CCA(:ACC	ACA CTG	acata I <u>atai</u> Catci	GACG	GAGA CAGC CAA	GCA CGC TGG	TGT. CGA ACG	ATG AAAG TGA CCG	AGT ATG ACT TAG	atg. GCT. TTA	ATP ATT ATT TGC TGP	GAA CCT	TAC: GAT TGT AAA	GGT.	<u>CAI</u> AGI	'TAI ATGC	ICT IGT CCT	3GG IAG ITC AGC
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S E TGGAG AGACC TGCAC CTACC	TCAA V TGAA ACTI TCCI	A AAGC K XTGG CGC.	? S GATA A I FCAC TCAC TCATC	R GACCO D ACACO CAAN GTCA GTCA	S GAA TZ E** CCATC CTTT7 AGC/	T AACT ** GGAG AATC AATC	T F TTGC TTTTI CTGC GCTC	CATI CATI CTTGC CTTT CACI	rtgi catti rgto rtgi	IGAA GAAA ICCC	agt. CAT TCA TTC	aga tct tgc gct cgc	GGGC GGGA ATA GAC. GAG	GAG AAG. TGT AAT	CCGGC ATGA CTTC CCAC	TAAAC CACC	CATC CATC CTTR GGTAT	<u>AA</u> G C <u>AT</u>	TTTT.	ACATA TATAT CATCT CCTTC ACGGA	GACC ATAC CCAI AGCC TCAI	CAA CAA CAA CAA	GCA CGC TGG CCT ACG	TGT. CGA ACG CGG	ATG AAG TGA CCG TGT	AGT ATG ACT TAG	ATG. GGCT. TTA GCTT	ATP ATI TGC TGP	GAA CCCT CCCC	GAT GAT TGT AAAJ GCT	GGT. CCT	<u>CAI</u> AGI	TAT TAT CTG CTG	ICT IGT CCT	2GG IAG ITC AGC
S E TGGAG AGACC TGCAC CTACC	TCAA V TGAA ACTI TCCI	A AAGC K XTGG CGC.	? S GATA A I FCAC TCAC TCATC	R GACCO D ACACO CAAN GTCF	S GAA TZ E** CCATC CTTT7 AGC/	T AACT ** GGAG AATC AATC	T F TTGC TTTTI CTGC GCTC	CATI CATI CTTGC CTTT CACI	rtgi catti rgto rtgi	IGAA GAAA ICCC	agt. CAT TCA TTC	aga tct tgc gct cgc	GGGC GGGA ATA GAC. GAG	GAG AAG. TGT AAT	CCGGC ATGA CTTC CCAC	TAAAC CACC CGTC	CATC CATC CTTR GGTAT	<u>AA</u> G C <u>AT</u>	TTTT. ACA CTG CTG	ACATA TATAT CATCT CCTTC ACGGA	GACC ATAC CCAI AGCC TCAI	CAA CAA CAA CAA	GCA CGC TGG CCT ACG	TGT. CGA ACG CGG	ATG AAG TGA CCG TGT	AGT ATG ACT TAG	ATG. GGCT. TTA GCTT	ATP ATI TGC TGP	GAA CCCT CCCC	GAT GAT TGT AAAJ GCT	GGT. CCT	<u>CAI</u> AGI	TAI TAI CTGC	ICT IGT CCT	2GG IAG ITC AGC
S E TGGAG AGACC TGCAC CTACC CGATA	TCAA V TGAA ACTT TCCT	A AGC K K CCCC. AAAA GCAT	SATA A I FCAC AATT ACTT CATC	R GACCO D ACACO CAATI CGTCF CGTCC ACGCO	S SAA TI E * CATO TTTT/ AGC/ SACAO	T AACT ** 666A6 AATC ACCT ACCT	TTGC TTTGC TTTT CTTT CTGC CTGC CAGC	CATT CATT TTGC TTGC CACT	PTGZ PTGZ PGTC PTGT PTGT PCAZ	IGAA GAAA FCCC ACCAT	agt. CAT TCA GCC GCT	AGA TCT TGC GCT CGC	GGGC GGA ATA GAC GAG	GAG AAG. TGT AAT	CGGG ATGA CTTC CCAC	AAAC CGTC	CATC CCTTC CCTTC CCTCA CCTCA	AAG	ACA CTG CTG CTG	ACATA IATAI CATCI CCTTC ACGGA	GACC ATAC CCAI AGCC TCAI	CAGC CAA CAAA CAAA	GCA CGC TGG CCT ACG	TGT. CGA ACG CGG CGG CAC	AAG AAG TGA CCCG TGT CGT	AGT ATG ACT TAG ACA	atg. GCT. TTA CTT TCT	ATA ATA ATI TGC TGA CCA GGG	.cac .cac .ccc .ccc	TAC: GAT(TGT) AAAA GCT(GAT(GGT. CCT CAT	<u>CAI</u> AGI CCA CCTG CCTG	TAT TAT CCTC CCCC	ICT IGT CCT CCC	GGG IAG ITC AGC ITT
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AGAGG S E AGGACC TGCAC CCTACC CCGATA	TCAA V TGAA ACTT TCCT ACAA	A AGC K K CCCC. AAAA GCAT	SATA A I FCAC AATT ACTT CATC	R GACCO D ACACO CAATI CGTCF CGTCC ACGCO	S SAA TI E * CATO TTTT/ AGC/ SACAO	T AACT ** 666A6 AATC ACCT ACCT	TTGC TTTGC TTTT CTTT CTGC CTGC CAGC	CATT CATT TTGC TTGC CACT	PTGZ PTGZ PGTC PTGT PTGT PCAZ	IGAA GAAA FCCC ACCAT	agt. CAT TCA GCC GCT	AGA TCT TGC GCT CGC	GGGC GGA ATA GAC GAG	GAG AAG. TGT AAT	CGGG ATGA CTTC CCAC	AAAC CGTC	CATC CCTTC CCTTC CCTCA CCTCA	AAG	ACA CTG CTG CTG	ACATA IATAI CATCI CCTTC ACGGA	GACC ATAC CCAI AGCC TCAI	CAGC CAA CAAA CAAA	GCA CGC TGG CCT ACG	TGT. CGA ACG CGG CGG CAC	AAG AAG TGA CCCG TGT CGT	AGT ATG ACT TAG ACA	atg. GCT. TTA CTT TCT	ATA ATA ATI TGC TGA CCA GGG	.cac .ccct .ccc	TAC: GAT(TGT) AAAA GCT(GAT(GGT. CCT CAT	<u>CAI</u> AGI CCA CCTG CCTG	TAT TAT CCTC CCCC	ICT IGT CCT CCC	GGG IAG ITC AGC ITT
S E TGGAG AGACC TGCAC CTACC CGATA TTGTT	TCAA V TGAA ACTT TCCT ACAA ACGO GTAO	A NAGC K NTGG CCCC. NAAA SCAT	SATA SATA ICAC AATT ACTT CATC SGAC	R GACCO D ACACO CAATI CCATO ACCGC ACCGC GTAT	S E+1 CATC CATC CATC CATC CATC CATC	T AACT ** GGAG AATC ATGA ACGT TGTT	T E TTGC TTTGC TTTT CTGC CTGC CTGC CAGC CAG	CATI CATI TTGC TTTGC CTCC GACI GAGG	ITGA CATT IGTO ICAA SGGA	IGAA GAAA ICCCC ACAT IGAT	GCC GCC TTG	AGA TCT TGC GCT CGC CAC	GGC GGA ATA GAC GAC ACC	GAG AAG. TGT TCC. CCG AAG.	CGGC ATGA CTTC CCAC ACTA GGTC ATAI	, AAAAC CACC CGTC	CATC COTTO C	AAG	ACA CTG CTG CTG CTG CTG CTG CTG CCTG CCT	ACATP IATAI CATCI CCTTC ACGGA	GACC ATAC CCAI AGCC ITCAI GCCI GTCI	CAGC CAA CAAA CAAA CAAAA	GCA CGC TGG CCT ACG TTG	TGT. CGA ACG CGG CAC TTT GCG.	ATG AAAG TGA CCGG CGT AGG	AGT ATG ACT TAG ACA AGC	ATG. GGCT. TTA CTT TCT TCT-	ATA ATI TGC TGA GGC	GAA GAA CCCT CCCT	GAT GAT TGT AAAA GCT GAT AGG	GGT. CCT CAT CAT	<u>CAI</u> AGI CCA CCI CCI	TAI TAI CGCC CGCC	ICT IGT IGT IGC IGC IGG	SGG IAG ITC AGC ITT IGG ITA
S E .TGGAG .AGACC .TGCAC .CTACC .CGATA	TCAA V TGAA ACTT TCCT ACAA ACGO GTAO	A NAGC K NTGG CCCC. NAAA SCAT	SATA SATA ICAC AATT ACTT CATC SGAC	R GACCO D ACACO CAATI CCATO ACCGC ACCGC GTAT	S E++ CATC TTTT AGC/ GAG/	T AACT ** GGAG AATC ATGA ACGT TGTT	T E TTGC TTTGC TTTT CTGC CTGC CTGC CAGC CAG	CATI CATI TTGC TTTGC CTCC GACI GAGG	ITGA CATT IGTO ICAA SGGA	IGAA GAAA ICCCC ACAT IGAT	GCC GCC TTG	AGA TCT TGC GCT CGC CAC	GGC GGA ATA GAC GAC ACC	GAG AAG. TGT TCC. CCG AAG.	CGGC ATGA CTTC CCAC ACTA GGTC ATAI	, AAAAC CACC CGTC	CATC COTTO C	AAG	ACA CTG CTG CTG CTG CTG CTG CTG CCTG CCT	ACATP IATAI CATCI CCTTC ACGGA	GACC ATAC CCAI AGCC ITCAI GCCI GTCI	CAGC CAA CAAA CAAA CAAAA	GCA CGC TGG CCT ACG TTG	TGT. CGA ACG CGG CAC TTT GCG.	ATG AAAG TGA CCGG CGT AGG	AGT ATG ACT TAG ACA AGC	ATG. GGCT. TTA CTT TCT TCT-	ATA ATI TGC TGA GGC	GAA GAA CCCT CCCT	GAT GAT GAT GCT GAT AAA	GGT. CCT CAT CAT	<u>CAI</u> AGI CCA CCI CCI	TAI TAI CGCC CGCC	ICT IGT IGT IGC IGC IGG	SGG IAG ITC AGC ITT IGG ITA

(Bermudez-Torrez 1996). We conclude that the wild-type strain m567 is diploid or aneuploid but are unable to define whether the complete genome is doubled in DNA content or whether single chromosomes containing genes of the isoprenoid pathway are multicopy.

For gene copy number estimation of the HMGR gene, strains m567 and MBC31 were both analyzed. Genomic DNA was digested with several restriction enzymes and probed with the 1.05-kb *Bam*HI/*Sal*I fragment from λ 24/1. Digestion resulted in one distinct hybridization band with the exception of *Sph*I, which cuts once in the 1.05-kb *Bam*HI/*Sal*I fragment that was used as probe (Fig. 3). Both the wild-type strain and its haploidized derivative showed the same hybridization pattern suggesting that if strain m567 is not haploid it contains two or more identical copies of the HMGR gene.

Expression of the HMGR gene under different cultivation conditions

It is well known that gibberellin biosynthesis is inhibited by high amounts of glucose and ammonium (Brückner and Blechschmidt 1991; Brückner 1992), though the target genes involved are not known. To investigate whether expression of the HMGR gene is regulated at the transcriptional level, *G. fujikuroi* RNA was subjected to RNA-blot analysis. In a first experiment, expression of the HMGR gene was analyzed under conditions either inducing or repressing GA₃ production. *G. fuikuroi* m567 was incubated in a plant oil-production medium (avoiding glucose repression) with corn-steep liquor as a complex nitrogen source, and in a synthetic medium with glucose excess and ammonium, which reduces GA₃ amount about tenfold. After 3 days of growth, total RNAs were isolated from both mycelia and probed with the 4.2-kb *Eco*RI/*Sal*I fragment.

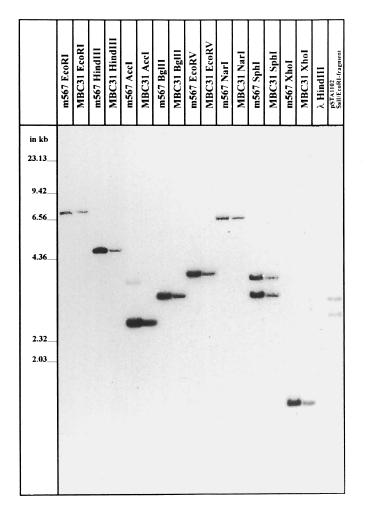
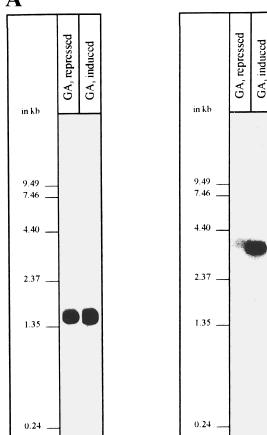


Fig. 3 Copy number estimation for the HMG-CoA reductase gene of *G. fujikuroi* in the genome of the wild-type strain m567 and the haploidized derivative MBC31. Genomic DNA of both strains was digested with different restriction enzymes and probed with the 1.05-kb *Bam*HI/*Sal*I fragment of pJ25

This experiment revealed a transcript size of approximately 3.0 kb corresponding to the size of the HMGR gene (Fig. 4 A). As shown in Fig. 4, the HMGR gene mRNA level is much higher under GA3-inducing conditions (plant oil and corn-steep liquor) than under GA3 repressing conditions (glucose as carbon source, ammonium excess). To ascertain if the increase of HMGR mRNA level is induced by plant components (corn-steep liquor and plant oil) or is due to C- or N-de-repression, G. fujikuroi m567 was grown in a minimal medium with sucrose and additional supplements (vitamins and trace elements) for 6 days. After washing, the mycelium was transferred to minimal media either containing a glucose excess or else lacking glucose (data not shown). In a second experiment, the mycelium was transferred to synthetic media with an NH⁺₄ excess or else lacking ammonia (Fig. 4 B). Total RNA was isolated from mycelia harvested at 0, 15, 30, 60, 120 and 300 min and probed with the Sall/BamHI fragment of pSTA1100. Under



B

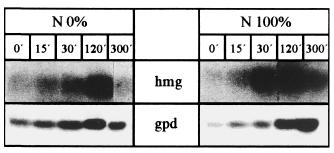


Fig. 4A RNA-blot analysis of the HMGR gene of *G. fujikuroi* m567 cultivated under conditions repressing GA₃ production (glucose excess) or stimulating GA₃ production (plant oil as carbon source, cornsteep liquor). The RNA was probed with the 4.2-kb *EcoRVSal* fragment of pJ25. Left part shows the hybridization of the same total RNA with the *C. purpurea gpd* gene (Jungehülsing et al. 1994). **B** RNA-blot analysis of the HMGR gene of *G. fujikuroi* m567 under conditions of ammonium excess (N 100%) and starvation (N 0%), respectively. Total RNA was isolated after 0, 15, 30, 60, 120 and 300 min and probed with the 1.05-kb *SalVBam*HI fragment of pJ25. Control hybridization was carried out with the *gpd* gene of *C. purpurea* (Jungehülsing et al. 1994)

all four conditions, with glucose or ammonia excess and glucose or ammonium starvation, no difference regarding the HMGR mRNA level could be detected between the different media. The HMGR transcript reached a maximum at 120 min; after that the transcript declined. This transient increase of transcript level could be the result of growth stimulation following the transfer into the new media after reaching the idiophase in pre-culture.

Therefore, the HMGR gene is expressed constitutively. The increased level of mRNA in plant-oil medium which is accompanied by an increased GA_3 level is not the result of C- or N-de-repression. GA_3 increase seems to be the consequence of a specific induction of the expression of the HMGR gene by plant components.

Comparison to the HMGR encoding gene of *S. manihoticola*

The ascomycete *S. manihoticola* is known to be a pathogen of cassava (*Cassava esculenta*) causing an enormous internodal growth of young infected cassava plants and as well as the production of plant tumors as a result of gibberellin formation by the fungus. It has been shown that GA₄ is the active principle of the plant disease (Rademacher and Graebe 1979; Zeigler et al. 1980). In contrast to *G. fujikuroi, S. manihoticola* is not able to synthezise 1,2-dehydroand 13-dehydroxy GAs like gibberellic acid GA₃ (Bearder 1983).

Nothing is known about the genetics and molecular genetics of GA synthesis in *S. manihoticola*. In order to compare the HMGR gene sequence data of the two GA-producing phytopathogenic ascomycetes, a part of the conserved C-terminal region of the *S. manihoticola* HMGR gene was isolated by PCR with the primer combination HMG 3/ HMG 4. The 840-bp *S. manihoticola* PCR fragment was subcloned into pCR-Script (Stratagene) and designated pSMHMG. This part of the *S. manihoticola* HMGR gene was sequenced and used for sequence comparisons.

In Table 2 the similarities of a 277 amino-acid fragment of the HMGR conserved region of *G. fujikuroi*, and other

Table 2 Comparison of the 277 amino acid HMGR PCR fragment of *S. manihoticola* (data bank accession number: X94308) with the homologous parts of some other HMGRs

Organism	Numbers of aminoacids	Similarity (%)	Identity (%)
S. manihoticola	1-277	100.0	100.0
G. fujikuroi	526-801	88.0	77.5
S. cerevisiae HMGR1	626-897	80.6	70.5
S. cerevisiae HMGR2	622-893	81.7	70.9
U. maydis HMGR1	698-988	80.4	63.8
M. auratus	466-739	73.3	59.4
A. thaliana HMGR1	173 - 446	70.0	53.2
H. brasiliensis HMGR3	173 - 446	72.3	53.2
D. melanogaster	490-763	69.9	53.9
S. mansoni	471 - 748	63.9	42.7
H. volcanii	1 - 281	58.0	37.5
P. mevalonii	1 - 268	42.7	21.7

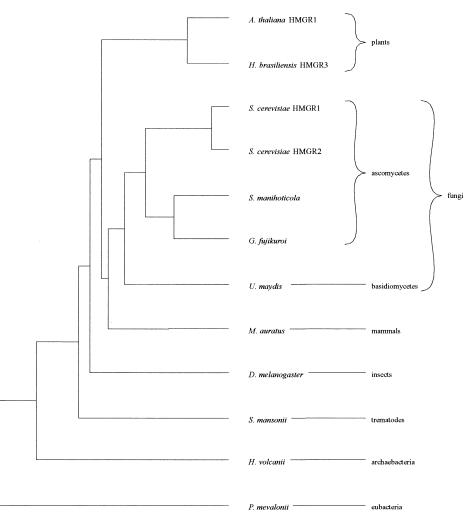
organisms, to the respective sequence of *S. manihoticola* are summarized. All ascomycetes share the greatest degree of similarity in this 277 amino-acid sequence, but the relationship between *G. fujikuroi* and *S. manihoticola* is the closest. This alignment was used for creating a phylogenetic dendrogram illustrating the evolutionary relationship of these HMG-CoA reductases (Fig. 5).

Estimation of HMGR gene copy number in *S. manihoticola* was carried out as described for *G. fujikuroi* m567. Genomic DNA of wild-type strain Lu949 was restricted with several enzymes and probed with the heterologous pSTA1100 *Sall/Bam*HI fragment and the homologous PCR fragment (the 840-bp *Kpnl/Sall* fragment of pSMHMG2). The two hybridization patterns were identical, indicating that *S. manihoticola*, like *G. fujikuroi*, possesses only one HMGR gene copy (data not shown).

Discussion

In this study, we described the isolation and characterization of the G.fujikuroi HMGR gene and the isolation of a part of the S.manihoticola HMGR gene by PCR. Both G. fujikuroi and S. manihoticola contain only one HMGR gene, as indicated by Southern-blot analysis. This is in contrast to S. cerevisiae (Basson et al. 1986), U. maydis (Croxen et al. 1994) and some zygomycetes (Burmester and Czempinsky 1994) in which two structural genes are present. It is not possible to determine the exact number of homologous HMGR gene copies which should exist in G. fujikuroi wild-type strain m567. This isolate seems to possess more than a haploid genome according to DNA content measurement, mutation experiments, and the measurement of GA_3 yield, both for this wild-type strain m567 and for the benomyl-treated derivative MBC31 (Bermudez-Torrez 1996).

The 2928-bp open reading frame of the HMGR gene of G. fujikuroi is interrupted by a putative 47-bp-long intron in position 2591 whereas the HMGR genes of S. cerevisiae and U. maydis do not posses introns. A comparison with the introns of the Arabidopsis thaliana HMGR gene1, the Hevea brasiliensis HMGR gene1 and HMGR gene3, and the Mesocricetus auratus HMGR gene revealed that intron position is not conserved among the HMGR genes of these organisms. The HMGR gene of G. fujikuroi encodes a predicted protein of 976 amino-acids which is shortened in the N-terminus in comparison to the other fungal HMGRs. The G. fujikuroi HMGR exhibits a greater overall similarity to U. maydis HMGR1 (64.3%) and S. cerevisiae HMGR2 (65.8%) than to the product of the S. cerevisiae HMGR gene1 (63.8%). The sequenced C-terminal part of the S. manihoticola HMGR gene product reveals that the highest homology can be found between the two pathogenic ascomycetes G. fujikuroi and S. manihot*icola*. The similarity in this part of the C-terminus is about 83%. This high sequence similarity within the catalytic domain of the G. fujikuroi HMG-CoA reductase to Ustilago and yeast gene products and to HMG-CoA reductases from Fig. 5 A phylogenetic tree based on the alignment of the amino-acid sequences shown in Table 2 was constructed using the program "Husar" (Heidelberg) (Sources of sequences: S. cerevisiae HMGR1, HMGR2: Basson et al. 1988; U. maydis HMGR: Croxen et al. 1994; M. auratus HMGR: Skalnik and Simoni 1985: A. thaliana: Learned and Fink 1989: Caelles et al. 1989: H. brasiliensis HMGR1 and HMGR3: Chye et al. 1991 b; D. melanogaster HMGR: Gertler et al. 1988; S. mansoni HMGR: Rajkovic et al. 1989; H. volcanii HMGR: Lam and Dollittle 1992; P. mevalonii HMGR: Beach and Rodwell 1989)



a wide range of other organisms indicates the high sequence conservation of this enzyme during evolution which may be associated with substrate and/or co-factor binding sites or catalytic activity.

The phylogenetic tree presented in Fig. 5 demonstrates that HMG-CoA reductase is helpful for analyzing the evolutionary relationships between different organisms. Protein structure and function are well conserved during evolution but the number of amino-acid exchanges which do not change the structure and the function of the enzyme give rise to a phylogenetic tree of general interest.

The N-terminal region of the *G. fujikuroi* HMG-CoA reductase gene contains seven putative hydrophobic domains using Kyte and Doolittle (1982) hydropathy plots (Sengstag et al. 1990). In *U. maydis* and *S. cerevisiae* eight hydrophobic regions were predicted but only seven have been recognized functionally as possible transmembrane domains (Sengstag et al. 1990). Besides anchoring into the endoplasmic reticulum, the membrane-spanning region has other functions. In mammalian cells, the membrane-bound portion of the protein is required for the sterol-regulated proteolytic degradation of the enzyme (Gil et al.

1985). The N-terminal region of the protein is also important in both *S. cervisiae* and mammalian cells for triggering increased membrane synthesis (Wright et al. 1988; Anderson et al. 1993).

Within the C-terminal domain some amino acids are functionally conserved and may play an important role in the structural conformation and/or in the catalytic properties of the enzyme. Two histidine and five cysteine residues are common to all HMGRs that have been analyzed (Caelles et al. 1989). Most of these residues are located within the more conserved region among the different HMG-CoA reductases, the so called b domains (b1 and b2) which have been postulated to be a part of the active site for hamster HMGR (Liscum et al. 1985). This region is especially rich in glycine and cysteine residues. Whereas the glycine residues are important in maintening the correct structure of the catalytic domains, the cysteine residues may play an important role in the catalytic process in addition to their function in structural maintenance (Dugan and Katiyar 1986). Amino acid residues which have already been shown to be important for catalysis in the HMGRs of P. mevalonii and hamster include the histidine

residue at position 924 (Darnay et al. 1992; Darney and Rodwell 1993) and the glutamate and aspartate residues at positions 618 (domain b1) and 828 (domain b2), respectively (Wang et al. 1990; Frimpong and Rodwell 1994). Of particular interest, with respect to the regulation of HMG-CoA reductase activity, is the serine residue at position 930. This residue is located close to the second catalytically important histidine residue at position 924. In eukaryotic HMGRs, this serine residue is known to be phosphorylated by AMP-activated proteine kinase, resulting in a loss of catalytic activity (Clark and Hardie 1990; Gillespie and Hardie 1992; Sato et al. 1993). Interestingly, neither of the S. cerevisiae HMGRs contain a serine residue at this position in the protein. The deduced amino-acid sequence of the C-terminal part of S. manihoticola HMGR possesses all conserved amino acids of domain b1 and all glycine and cysteine residues common to b1 and b2.

It is known that gibberellin biosynthesis is regulated via N- and C-catabolite repression (Brückner and Blechschmidt 1991; Brückner 1992). However, the target gene(s) for those regulation mechanisms are unknown.

The promotor region of 769 bp of the HMG-CoA reductase gene of *G. fujikuroi* was analyzed with respect to the known consensus sequences for DNA-binding proteins of C- and N-regulation. Five GATA motifs were found in the promotor region, three of them lay together in a 18-bp region next to the putative TATA box. Haas and Marzluf (1995) postulated that two or three such GATA elements must be located within a 100-bp region in order to be involved in N-metabolite regulation. A putative binding site for a glucose regulator protein was found 711 bp upstream of the translation initiation site, which is similar to the binding site for CREA of the amd S gene of *A. nidulans* (Davis et al. 1993): 5'-CCCCGGACT-3'.

The existence of such consensus sequences indicates the possible regulation of this primary metabolism gene. However, the expression analysis for the HMGR gene revealed an opposite result. Excess of glucose or ammonium does not have repressing effects on HMGR gene expression. Therefore, the HMGR gene is regulated like a housekeeping gene. Because of the importance of the gene for membrane synthesis and for cell growth, this result is not surprising. On the other hand, the HMGR gene seems to be induced by plant components, perhaps reflecting the close relationship between gibberellin biosynthesis and plant disease.

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References

Anderson RGW, Orci L, Brown MS, Garcia-Segura LM, Goldstein JL (1993) Ultrastructural analysis of crystalloid endoplasmatic reticulum in UT-1 cells and its disappearance in response to cholesterol. J Cell Sci 63:1–20

- Basson ME, Thorsness M, Rine J (1986) Saccharomyces cerevisiae contains two functional genes encoding 3-hydroxy-3-methylglutaryl-coenzyme A reductase. Proc Natl Acad Sci USA 83: 5563–5567
- Basson ME, Thorsness M, Finer-Moore J, Stroud RM, Rine J (1988) Structural and functional conservation between yeast and human 3-hydroxy-3-methylglutaryl coenzyme A reductases, the rate limiting enzyme of sterol biosynthesis. Mol Cell Biol 8:3797– 3808
- Beach MJ, Rodwell VW (1989) Cloning, sequencing and overexpression of *mvaA*, which encodes *Pseudomonas mevalonii* 3-hydroxy-3-methylglutaryl coenzyme A reductase. J Bacteriol 171: 2994–3001
- Bearder JR (1983) In vivo diterpenoid biosynthesis in *Gibberella fujikuroi*: the pathway after ent-kaurene. In: Crozier A (ed) The biochemistry and physiology of gibberellins. Vol.1. Praeger Publishers, New York, pp 251–388
- Bermudez-Torrez K (1996) Methoden der Stammentwicklung am Beispiel des Gibberellinbildners Gibberella fujikuroi. Dissertation, Westfälische Wilhelms-Universität, Münster
- Brückner B (1992) Regulation of gibberellin formation by the fungus Gibberella fujikuroi. In: Secondary metabolites: their function and evolution. Wiley, Chichester, Ciba Foundation Symposium 171:129–143
- Brückner B, Blechschmidt D (1991) The gibberellin fermentation. Crit Rev Biotechnol 11:163–192
- Burmester A, Czempinski K (1994) Sequence comparison of a segment of the gene for 3-hydroxy-3-methylglutaryl-coenzyme A reductase in zygomycetes. Eur J Biochem 220:403–408
- Caelles C, Ferrer A, Bacells L, Hegardt FG, Boronat A (1989) Isolation and structural characterization of a cDNA encoding *Arabidopsis thaliana* 3-hydroxy-3-methylglutaryl coenzyme A reductase. Plant Mol Biol 13:627–638
- Cenis JL (1992) Rapid extraction of fungal DNA for PCR amplification. Nucleic Acids Res 20:2350
- Chambers JAA, Russo VEA (1986) Isolating RNA is easy and fun. Fungal Gen Newslett 33:22–23
- Chin DJ, Gil G, Russell DW, Liscum L, Luskey KL, Basu SK, Okayama H, Berg P, Goldsetin JL, Brown MS (1984) Nucleotide sequence of 3-hydroxy-3-methylglutaryl coenzyme A reductase, a glycoprotein of the endoplasmic reticulum. Nature 308:613–617
- Church GM, Gilbert W (1984) Genomic sequencing. Proc Natl Acad Sci USA 81:1991–1995
- Chye M-L, Kush A, Tan C-T, Chua N-H (1991 a) Characterization of cDNA and genomic clones encoding the 3- regulatory gene of *N. crassa.* Mol Gen Genet 214:74
- Chye M-L, Tan C-T, Chua N-H (1991 b) Three genes encode 3-hydroxy-3-methylglutaryl-coenzyme A reductase in *Hevea brasiliensis: hmg*1 and *hmg*3 are differentially expressed. Plant Mol Biol 19:473–484
- Clarke PR, Hardie DG (1990) Regulation of HMG-CoA reductase: identification of the site phosphorylated by the AMP-activated protein kinase in vitro and in intact rat liver. EMBO J 9:2439– 2446
- Croxen R, Goosey MW, Keon JPR, Hargreaves JA (1994) Isolation of an *Ustilago maydis* gene encoding 3-hydroxy-3-methylglutaryl-coenzyme A reductase and expression of a C-terminaltruncated form in *Escherichia coli*. Microbiology 140: 1363–2370
- Darnay BG, Rodwell VW (1993) His⁸⁶⁵ is the catalytically important histidyl residue of Syrian hamster 3-hydroxy-3-methylglutaryl-coenzyme A reductase. J Biol Chem 268:8429–8435
- Darnay BG, Wang Y, Rodwell VW (1992) Identification of the catalytically important histidine of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. J Biol Chem 267:15064–15070
- Davis MA, Kelly JM, Hynes MJ (1993) Fungal catabolic gene regulation: molecular genetic analysis of the *amdS* gene of *Aspergillus niger*. Genetica 90:133–145
- Dugan RE, Katiyar SS (1986) Evidence for catalytic site cysteine and histidine by chemical modification of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. Biochem Biophys Res Commun 141:278–28

- Frimpong K, Rodwell VW (1994) Catalysis by Syrian hamster 3-hydroxy-3-methylglutaryl-coenzyme A reductase: proposed roles of histidine 865, glutamate 558 and aspartate 766. J Biol Chem 259:11478–11483
- Fu YH, Marzluf GA (1990) nit-2, the major nitrogen regulatory gene of Neurospora crassa encodes a protein with a putative zinc finger DNA-binding domain. Mol Cell Biol 10:1056–1065
- Gertler FB, Choi-Ying C, Richter-Mann L, Chin DJ (1988) Developmental and metabolic regulation of the *Drosophila melanogaster* 3-hydroxy-3-methylglutaryl coenzyme A reductase. Mol Cell Biol 8:2713–2721
- Gil G, Faust JR, Chin DJ, Goldstein JL, Brown MS (1985) Membrane-bound domain of HMG CoA reductase is required for sterol-enhanced degradation of the enzyme. Cell 41:249–259
- Gillespie JG, Hardie DG (1992) Phosphorylation and inactivation of HMG-CoA reductase at the AMP-activated protein kinase site in response to fructose treatment of isolated rat hepatocytes. FEBS Lett 306:59–62
- Graebe JE (1987) Gibberellin biosynthesis and control. Annu Rev Plant Physiol 38:419–465
- Gurr SJ, Unkles SE, Kinghorn JR (1987) The structure and organization of nuclear genes in filamentous fungi. In: Kinghorn JR (ed) Gene structure in eucaryotic microbes. IRL Press, Oxford Washington, pp 93–139
- Haas H, Marzluf GA (1995) NRE, the major nitrogen regulatory protein of *Penicillium chrysogenum*, binds specifically to elements in the intergenic promotor regions of nitrate assimilation and penicillin biosynthetic gene clusters. Curr Gen 28:177–183
- Jungehülsing U, Arntz C, Smit R, Tudzynski P (1994) The Claviceps purpurea glyceraldehyde-3-phosphate dehydrogenase gene: cloning, characterization, and use for the improvement of a dominant selection system. Curr Genet 25:101–106
- Kudla B, Caddick MX, Langdon T, Martinez-Rossi N, Bennett CF, Sibley S, Davies RW, Arst Jr HN (1990) The regulatory gene areA mediating nitrogen metabolite repression in A. nidulans. Mutations affecting specificity of gene activation alter a loop residue of a putative zinc finger. EMBO J 9:1355–1364
- Kyte J, Doolittle RF (1982) A simple method for displaying the hydropathic character of a protein. J Mol Biol 157:105–132
- Lam WL, Doolittle WF (1992) Mevinolin-resistant mutations identify a promoter and the gene for a eukaryotic-like 3-hydroxy-3methylglutaryl-CoA reductase in the Archaebacterium Haloferax volcanii. J Biol Chem 267:5829–5834
- Learned RM, Fink GR (1989) 3-hydroxy-3-methylglutaryl coenzyme A reductase from Arabidopsis thaliana is structurally distinct from the yeast and animal enzymes. Proc Natl Acad Sci USA 86:2779–2783

- Liscum L, Finer-Moore J, Stroud RM, Luskey KL, Brown MS, Goldstein JL (1985) Domain structure of 3-hydroxy-3-methylglutaryl coenzyme A reductase, a glycoprotein of the endoplasmic reticulum. J Biol Chem 260:522–530
- Luskey KL, Stevens B (1985) Human 3-hydroxy-3-methylglutaryl coenzyme A reductase. J Biol Chem 260:10271–10277
- Rademacher W, Graebe JE (1979) Gibberellin A₄ produced by Sphaceloma manihoticola, the cause of the super-elongation disease of cassava (Manihot esculenta). Biochem Biophys Res Commun 91:35–40
- Radley M (1956) Occurences of substances similar to gibberellic acid in higher plants. Nature 178:1070–1071
- Raijkovic A, Simonsen JN, Davis RE, Rottman FM (1989) Molecular cloning and sequence analysis of 3-hydroxy-3-methylglutaryl-CoA reductase from the human parasite *Schistosoma mansoni*. Proc Natl Acad Sci USA 86:8217–8221
- Sambrook J, Fritsch EF, Maniatis TM (1989) Molecular cloning: a laboratory manual. Cold Spring Harbour Laboratory, Cold Spring Harbour, New York
- Sato R, Goldstein JL, Brown MS (1993) Replacement of serine-871 of hamster 3-hydroxy-3-methylglutaryl CoA reductase prevents phosphorylation by AMP-activated kinase and blocks inhibition of sterol synthesis induced by ATP depletion. Proc Natl Acad Sci USA 90:9261–9265
- Sengstag C, Stirling C, Schekman R, Rine J (1990) Genetic and biochemical evaluation of eucaryotic membrane protein topology: multiple transmembrane domains of *S. cerevisiae* 3-hydroxy-3-methylglutaryl coenzyme A reductase. Mol Cell Biol 10:672– 680
- Skalnik DG, Simoni RD (1985) The nucleotide sequence of Syrian hamster HMG-CoA reductase cDNA. DNA 4:439-444
- Unkles SE (1992) Genome organization in industrial filamentous fungi. In: Turner G (eds) Applied molecular genetics of filamentous fungi. Kinghorn JR, Blackie Academic and Professional, London Glasgow, pp 28–53
- Wang Y, Darnay BG, Rodwell VW (1990) Identification of the principal catalytically important acidic residue of 3-hydroxy-3-methylglutaryl coenzyme A reductase. J Biol Chem 265:21634–21641
- Wright R, Basson M, D ari L, Rine J (1988) Increased amounts of HMG-CoA reductase induce 'Karmellae': a proliferation of stacked membrane pairs surrounding the yeast nucleus. J Cell Biol 107:101–114
- Yabuta T (1935) Biochemistry of the "bakanae" fungus of rice. Agric Hort 10:17–22
- Zeigler RS, Powell LE, Thurston, HD (1980) Gibberellin A₄ production by *Sphaceloma manihoticola*, causal agent of cassava superelongation disease. Phytopathology 70:589–593