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ORIGINAL PAPER

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Cloning and characterization of the gene encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase in melon (*Cucumis melo* L. *reticulatus*)

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Abstract We have isolated a cDNA for *Cm-HMGR*, encoding 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase in melon (*Cucumis melo* L. *reticulatus*; Genbank Accession No. AB021862). *Cm-HMGR* encodes a polypeptide of 588 amino acids that contains two transmembrane domains and a catalytic domain. Database searches revealed that Cm-HMGR shows homology to HMG1 (63.7%) and HMG2 (70.3%) of tomato, to HMG1 (77.2%) and HMG2 (69.4%) of *Arabidopsis thaliana*, and to *HMGR* of tobacco (72.6%). Functional expression in a HMG-CoA reductase-deficient mutant yeast showed that *Cm-HMGR* products mediate the synthesis of mevalonate. Northern analysis revealed that the level of *Cm-HMGR* mRNA in the fruit increased after pollination and markedly decreased at the end of fruit enlargement. During ripening, *Cm-HMGR* mRNA levels increased markedly in the fruit. In parallel with mRNA expression, Cm-HMGR activity increased after pollination, whereas no Cm-HMGR activity was detectable during fruit ripening. Our results suggest that *Cm-HMGR* is important during early post-pollination development of the fruit in melon.

Key words Melon · 3-Hydroxy-3-methylglutaryl coenzyme A reductase gene · Fruit development · Yeast complementation

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Introduction

A leading issue in the development of higher plants is the determination of fruit size. To elucidate the mechanism responsible, Higashi and colleagues (1999) observed the histology of cells during fruit development in two melon genotypes whose mature fruits differed in size. The results suggested that the degree of cell proliferation during early fruit development determines fruit size in melon and that temperature affects the factor that regulates the extent of cell proliferation. However, the precise mechanism by which cell proliferation is regulated remained unclear.

Using tomato, Narita and Gruissem (1989) demonstrated that expression and activity of the 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) encoded by *HMG1* are required during early fruit development. Histochemical analysis of transgenic tomato strains carrying *HMG1::GUS* reporter gene fusions, as well as of the transcription of *HMG1* and the activity of HMGR in suspensions of tomato cells, suggested that a primary role of *HMG1* is to meet the demand for mevalonate that is associated with cell division and growth (Jelesko et al. 1999). In addition, Cowan et al. (1997) used normal and small-fruit phenotypes of avocado to probe the interaction between the end products of isoprenoid biosynthesis and the activity of HMGR in the metabolic control of fruit growth. The results suggested that accumulation of abscisic acid down-regulates mesocarpal HMGR activity and decreases fruit size by reducing the number of cells. These findings in tomato and avocado indicate the involvement of HMGR in fruit development.

End products of isoprenoid synthesis are very important in many biological processes in plants, such as the synthesis of membrane sterols and plant growth hormones (cytokinin, abscisic acid, gibberellins, and brassinosteroids), electron transport (cytochrome *a*, quinones, chlorophyll), isoprenylation of proteins, and production of sesquiterpenoid phytoalexins, which

provide resistance to pathogens (Chappell 1995; McGarvey and Croteau 1995; Weissenborn et al. 1995; Westwood et al. 1998). Mevalonate is essential for isoprenoid synthesis in plants, and HMGR is the key enzyme of isoprenoid synthesis, catalyzing the conversion of HMG-CoA into mevalonate.

HMGR genes already have been isolated from many plant species, such as *Arabidopsis* (Caelles at al. 1989; Learned and Fink 1989; Enjuto et al. 1994), potato (Choi et al. 1992; Bhattaharyya et al. 1995; Korth et al. 1997), tomato (Park et al. 1992), *Nicotiana sylvestris* (Genchik et al. 1992), para rubber tree (*Hevea brasilensis*; Chye et al. 1991, 1992), *Catharauthus roseus* (Maldonado-Mendoza et al. 1992), wheat (Aoyagi et al. 1993), cotton (Loguercio et al. 1999), and mulberry (Jain et al. 2000). The structure of plant HMGRs differs markedly from that in animals and fungi. The HMGRs found in animal and fungi have seven membrane-spanning domains in the N-terminal domain (Basson et al. 1988), whereas HMGRs isolated from higher plants usually have only two membrane-spanning domains (Learned and Fink 1989). Regardless of the number of membrane-spanning regions, HMGR is membrane bound (Chye et al. 1992).

In the present study, we isolated and characterized a gene encoding HMGR from melon and analyzed its transcription during fruit development. In addition, we discuss the possible role of melon HMGR in fruit growth.

Materials and methods

Plant material and growth

We used two cultivars of melon (*Cucumis melo* L. *reticulatus* cv. Fuyu A and Natsu 4) and their hybrid, Fuyu A × Natsu 4. For surface sterilization, melon seeds were soaked in 70% (v/v) ethanol for 15 s then in 1% (v/v) hypochlorite solution for 15 min; treated seeds were rinsed three times with distilled water. For RT-PCR, surface sterilized Fuyu A seeds were germinated on Murashige and Skoog (1962) medium (pH 5.8) with 3% (w/v) sucrose and 0.4% (w/v) gellan gum (Gelrite, Wako Pure Chemicals, Osaka, Japan) and grown in darkness at 25 °C for 10 days. The seedlings were harvested, then frozen in liquid nitrogen and stored at −80 °C until use. For other applications, seeds of the three genotypes were sown and grown in greenhouses. Freshly opened female flowers were hand-pollinated, and one fruit per plant was allowed to develop. Fruit was harvested at various times after pollination, and the pericarp tissue was separated from the contents of the seed cavity, sliced, frozen in liquid nitrogen, and stored at −80 °C until use.

Cloning of a *HMGR* homolog from a melon cDNA library

During a screen for *ETR* homologs in melon, we serendipitously isolated a clone encoding a protein that was highly homologous to other plant HMGRs (Sato-Nara et al. 1999) from a cDNA library generated from RNA isolated from melon fruits 50 days post-pollination. The cDNA fragment was subcloned in pBSII SK+ (Clontech, Palo Alto, Calif., USA). The inserts were sequenced with a 373S DNA sequencing system and ABI PRISM Dye Primer Cycle Sequencing-Ready Reaction Kits (Perkin-Elmer Cetus, Norwalk, Conn., USA). We used Genetix-Mac version 7.3 (Software Development Co., Tokyo, Japan) to analyze sequences.

Yeast growth and transformation

We used the *Saccharomyces cerevisiae* strain JRY2394 (*MAT***a**, *ade2*, *his3*, *met-*, *ura3*, *hmg1*, *hmg2*; kindly provided by Dr. Jasper Rine, U.C. Berkeley, Calif., USA) to investigate the function of *Cm-HMGR*. The yeast strain has loss-of-function mutations in two HMGR genes, *HMG1* and *HMG2*, and requires mevalonate for growth. The wild-type yeast strain TM 100 (*ura3, leu2, trp1*) was used as a control.

We cloned the *Cm-HMGR* cDNA into the pYES2 expression vector (Invitrogen) and transformed JRY2394 as described by Kaiser et al. (1994). The empty pYES2 vector was used as a control. *Cm-HMGR* transformants were selected on YM galactose medium containing 0.67% yeast nitrogen base without amino acids, 2% galactose, and 30 mg/l each of adenine, histidine, and methionine. For non-selective conditions, we added 5 mg/l mevalonate and 2% glucose instead of galactose.

Genomic Southern analysis

We isolated genomic DNA from young leaves of Fuyu A, Natsu 4, and the Fuyu A × Natsu 4 hybrid according to Wagner et al. (1987). Digested DNA (20-μg aliquots) was electrophoresed in a 0.8% agarose gel and transferred to Hybond-N+ (Amersham-Pharmacia Biotech, Tokyo, Japan). Using a 1.25-kb *Sal*I-*Bgl*II fragment encoding the catalytic domain as a probe, hybridization was performed as described by Church and Gilbert (1984) at 60 °C (low stringency) or 65 °C (high stringency) for 16 h. Results were visualized by autoradiography at −80 °C.

Northern analysis

We extracted total RNA from melon fruit at various developmental stages according to the protocol of Sato-Nara et al. (1999). We followed the method of Collart and Oliviero (1993) to isolate total RNA from yeast. We fractionated 10 μg of the total RNA on a 1.0% agarose/formaldehyde gel (Berk and Sharp 1978), and transferred the RNA to Hybond-N+ (Amersham). Using the 1.25-kb *Sal*I-*Bgl*II fragment encoding the catalytic domain as a probe, Northern hybridization was performed overnight at 65 °C in a solution containing 10% sodium dextran sulfate, 1% SDS, 1 M sodium chloride, 100 μg/ml denatured salmon sperm DNA, and the ^{32}P-labeled probe. After hybridization, the membranes were rinsed twice for 15 min each in 2 × SSC at room temperature and once with 2 × SSC containing 1% SDS for 30 min at 65 °C. The filters then were autoradiographed at –80 °C.

HMG-CoA reductase assay

To isolate microsomal membranes, pericarp was pulverized in liquid nitrogen, and 10 g of the powdered pericarp was homogenized in 40 ml of extraction buffer (10 mM TRIS-HCl pH 7.0, 0.35 M sucrose, 30 mM EDTA pH 8.0, and 10 mM β-mercaptoethanol). The homogenate was filtered through two layers of cheesecloth and centrifuged at 12,000 ×*g* for 10 min; the resulting supernatant was centrifuged at 50,000*g* for 60 min. The pellet was resuspended in a medium containing 0.2 M potassium phosphate (pH 6.9) and 25 mM DTT and the resulting suspension was used to assay HMGR activity. Yeast protein extract was prepared according to the method of Basson et al. (1987); protein concentration was determined by using the Protein Assay kit (Bio-Rad, Hercules, Calif., USA).

We used the radiometric assay of Oba et al. (1985), with slight modifications, to measure HMGR activity. The reaction mixture contained 1 μl of 0.2 M DTT, 2 μl of an NADPH-generating system (20 mM NADP, 100 mM glucose 6-phosphate and 0.01 U/μl glucose 6-phosphate dehydrogenase), 3 μl of [3-^{14}C]HMG-CoA (0.34 nM/μl, 740 kBq/ml, Amersham) and an amount of microsomal fraction equivalent to 10 μg protein in a total volume of 20 μl. The reaction was started by adding [3-^{14}C]HMG-CoA, and was stopped after 20 min incubation at 30 °C by adding 2 μl of 1 M MVA and 2 μl of 6 M HCl. Further incubation at 25 °C for 15 min

to ensure lactonization. After centrifugation, 10 µl of the supernatant solution was applied to a TLC plate (25 TLC aluminium sheets 20 × 20 cm, Silica gel 60, Merck), and developed with chloroform-acetone (2:1 v/v). The plate was exposed to iodine vapor to visualize mevalonolactone. Radioactivity was measured using a Storm860 Fluorescence Imager with Image Quant software (Molecular Dynamics, Sunnyvale, Calif., USA). One unit of HMG-CoA reductase activity was defined as the amount of enzyme that formed 1 nmol of mevalonate in 1 h at 30 °C.

Results

Molecular cloning of *Cm-HMGR*

We isolated a 2357-bp cDNA clone corresponding to the *HMGR* gene from a cDNA library prepared with RNA isolated from mature melon fruits. The deduced amino acid sequence (588 residues) showed homology to tomato HMG1 (63.7%) and HMG2 (70.3%), to *Arabidopsis* HMG1 (77.2%) and *Arabidopsis* HMG2 (69.4%), and was 72.6% homologous to tobacco HMGR (Fig. 1). Like other HMGRs from plants, the deduced protein has two predicted membrane-spanning domains, which are localized in the N-terminal region, and b1 and b2 domains, which represent the putative catalytic domains. Therefore we designated the corresponding gene *Cm-HMGR* (for *C. melo* 3-*h*ydroxy-3-*m*ethylglutaryl CoA *r*eductase; Genbank Accession No. AB021862).

Phylogenic analysis showed that plant HMGRs separated into two or three groups, and HMGRs from the same or related plant species tend to belong to the same

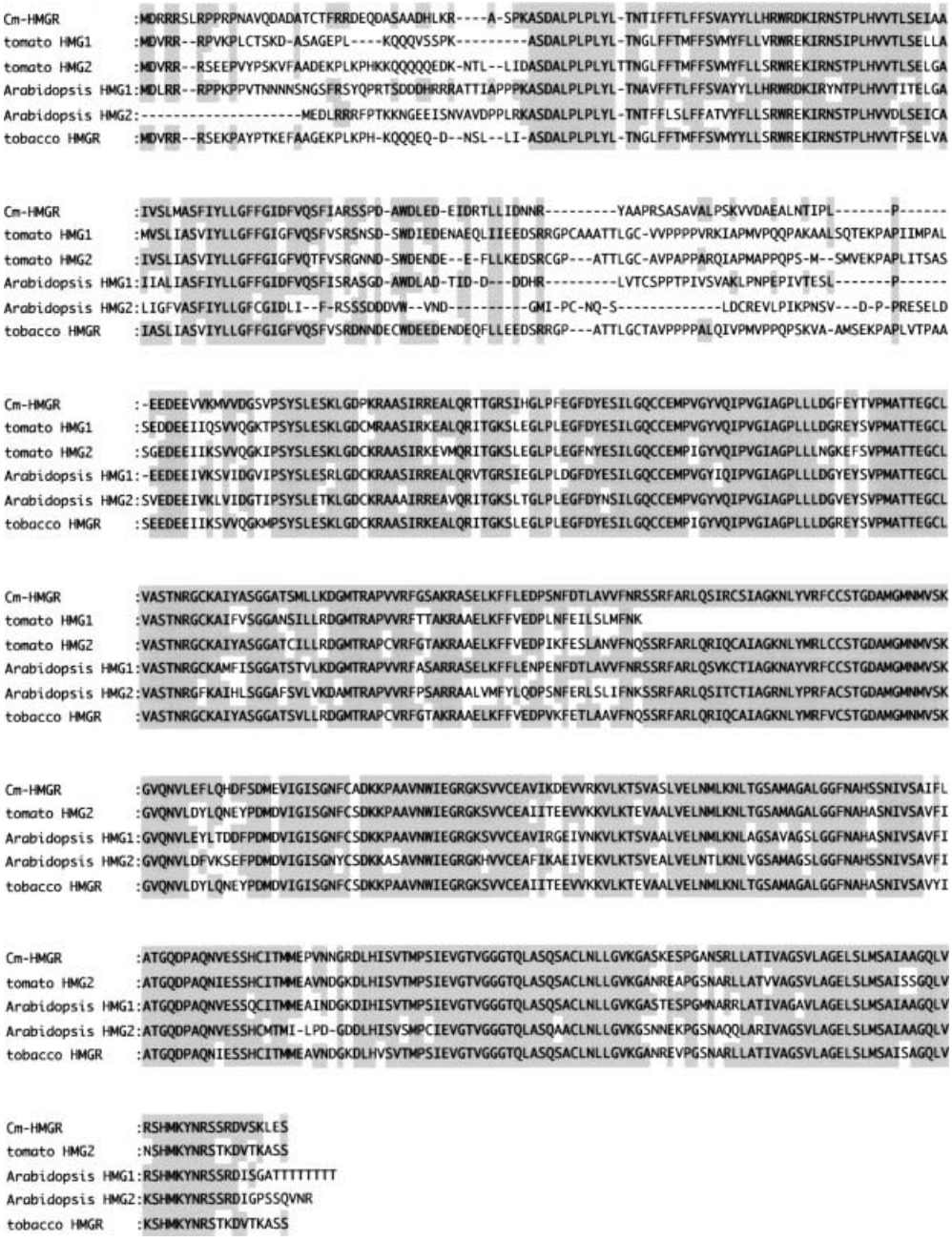

Fig. 1 Comparison of the 3-hydroxy-3-methylglutaryl-coenzyme A reductase from melon (Cm-HMGR), tomato, *Arabidopsis* and tobacco. The alignment was performed using GENETYX version 10.1 (SDC Software, Tokyo Japan)

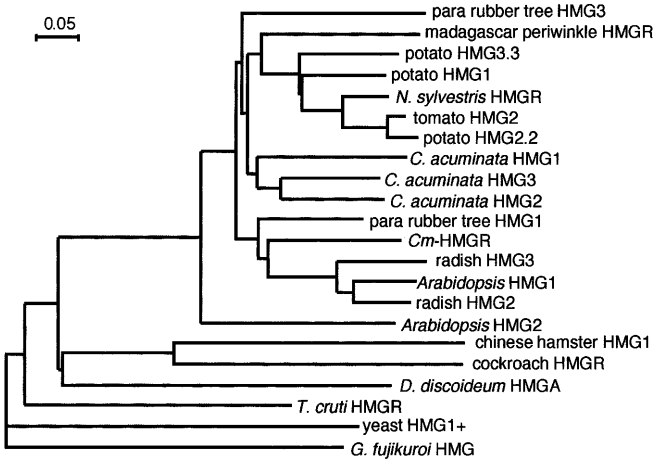

Fig. 2 Relationships between the primary amino acid sequences of HMGRs from various plants, animals, and fungi. The phylogenetic tree was constructed by using CLUSTAL X (Thompson et al. 1997). The number next to the node represents the bootstrap value from 1000 replicates. Deduced amino acid sequences were retrieved from the Genbank, EMBL and DDBJ databases. The Accession Nos. are: para rubber tree HMG3, U72145; Madagascar periwinkle HMGR, M96068; potato HMG3.3, U51986; potato HMG1, L01400; *Nicotiana sylvestris* HMGR, X63649; tomato HMG2, M69642; potato HMG2.2, U51985; *Camptotheca acuminata* HMG1, L10390; *C. acuminata* HMG3, U72145; *C. acuminata* HMG2, U72146; para rubber tree HMG1, X54659; Cm-HMGR, AB021862; radish HMG3, X68652; *Arabidopsis* HMG1, L19261; radish HMG2, X68651; *Arabidopsis* HMG2, L19262; Chinese hamster HMG1, L00165-L00183; cockroach HMGR, X70034; *Dictyostelium discoideum* HMGA, L19349; *Trypanosoma cruzi* HMGR, L78791; yeast HMG+, L76979; and *Gibberella fujikuroi* HMG, X94307

group (Fig. 2). CM-HMGR and tomato HMG2, both of which occur in developing fruit, belonged to different groups. Unfortunately, the complete sequence of tomato HMG1, another HMGR found in developing fruit, is not yet available. Therefore, we could not ascertain whether CM-HMGR and tomato HMG1 belong to the same group.

Functional analysis of *Cm-HMGR*

To analyze the function of Cm-HMGR, the cDNA was expressed in the yeast strain JRY2394, which lacks HMGR activity. The two *Cm-HMGR*-containing yeast strains HMG-Y4311 and HMG-Y4312 were selected on minimal medium containing galactose, and their growth was compared to that of JRY2394 and of the same strain carrying only the empty-vector (pYES2) (Fig. 3). All four strains were unable to grow on glucose-supplemented YM, but HMG-Y4311 and HMG-Y4312 grew on galactose-supplemented YM. In addition to the two *Cm-HMGR*-containing strains, the pYES2-transformed strain grew on YM supplemented with galactose and mevalonate; further addition of uracil enabled all four strains to grow.

Northern analysis confirmed that galactose strongly induced transcription of *Cm-HMGR* in HMG-Y4311 and HMG-Y4312 (Table 1). The HMGR activity assay showed that HMG-Y4311 and HMG-Y4312 produce functional HMGR. Both transgenic yeast lines had negligible levels of *Cm-HMGR* mRNA before induction, the levels of HMGR activity were markedly low, and these uninduced lines failed to grow in the absence of added mevalonate (data not shown). These results indicate that *Cm-HMGR* mRNA transcribed from the cloned cDNA insert encodes a functional HMGR protein.

Genomic Southern analysis

We used genomic Southern analysis to evaluate the genomic organization of *Cm-HMGR* in Fuyu A, Natsu 4, and their F1 hybrid. At high stringency, each of these samples was associated with a single, strong signal. At a low stringency, two weaker signals were found, in addition to the strong band in these lanes (Fig. 4). These results suggest that *Cm-HMGR* belongs to a small family of *hmgr* genes. In addition, the *Cm-HMGR*

Fig. 3 Complementation for growth of the yeast strain JRY2394, which lacks HMGR activity, by the *Cm-HMGR* cDNA. Growth of HMG-Y4311 and HMG-Y4312, which express Cm-HMGR, and the strain transformed with pYES2 is shown. For selection, the medium was supplemented with galactose to induce production of Cm-HMGR. The non-selective medium contained mevalonate and uracil

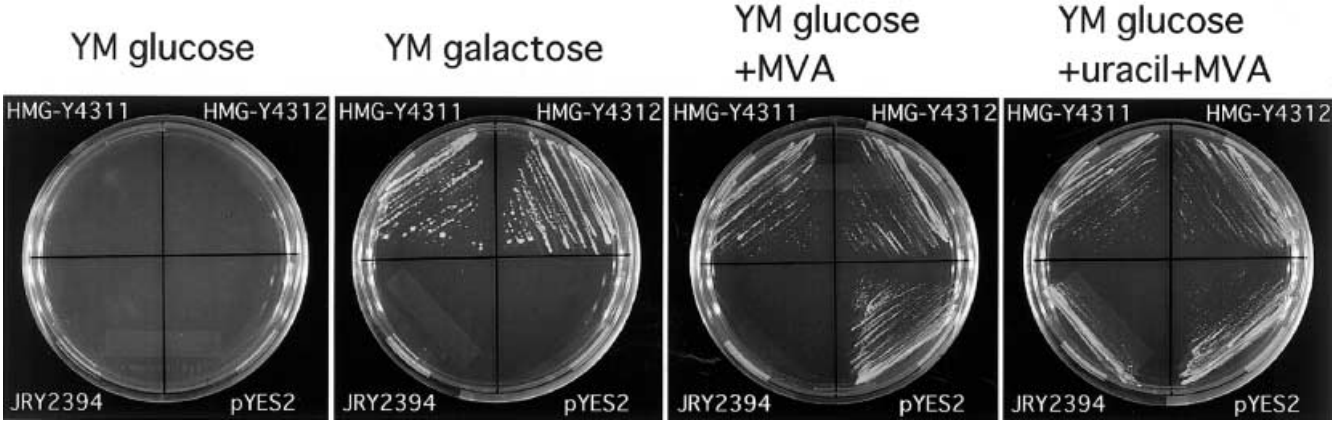

Table 1 HMGR activity of yeast lines transformed with *Cm-HMGR*

Yeast line	Medium	Plasmid	HMGR activity[a]	*HMGR* signal on Northern blot
HMG-Y4311	YM galactose	pYES::Cm-HMGR	68.98	Strong
HMG-Y4311	YM glucose + MVA	pYES::Cm-HMGR	0.09	Weak
HMG-Y4312	YM galactose	pYES::Cm-HMGR	56.28	Strong
HMG-Y4312	YM glucose + MVA	pYES::Cm-HMGR	0.09	Weak
JRY2394	YM glucose + MVA + Ura	pYES2	0.00	Not done
TM100	YM glucose + Ura + Lu – Trp	Not applicable	49.26	Not done

[a] HMGR activity is expressed in nmols of mevalonate (MVA) produced per h per mg HMGR protein

cDNA probe hybridized only with *Cm-HMGR* mRNA under high-stringency washing conditions.

Cm-HMGR in developing melon fruits

Fruit weights of Fuyu A, Natsu 4, and the hybrid Fuyu A × Natsu 4 (Fig. 5A) increased after pollination. The pattern of increasing fruit weight in Fuyu A fitted the logarithmic curve $y = 1341.0 \log(x) - 597.1$ ($r^2 = 0.84$); the equivalent curves for Natsu 4 and the F1 hybrid are given by the equations $y = 750.9 \log(x) - 316.9$ ($r^2 = 0.84$) and $y = 1070.8 \log(x) - 473.1$ ($r^2 = 0.85$), respectively. The fruit size of Fuyu A was larger than that of Natsu 4, and that of the hybrid was intermediate between Fuyu A and Natsu 4. These results suggest that the differences in fruit weight were controlled by genetic factors.

We used RNA blot hybridization to detect changes in the accumulation of *Cm-HMGR* transcripts during fruit development (Fig. 5B, D). During early fruit development in all three genotypes, *Cm-HMGR* transcripts accumulated rapidly during the first week after pollination, after which they decreased until reaching a minimum at 21 days after pollination. During the middle and late stages of fruit development (21 days after pollination and later), levels of *Cm-HMGR* transcripts in Fuyu A increased markedly, whereas in Natsu 4 and the hybrid they increased only slightly. More *Cm-HMGR* transcripts accumulated in the fruit of Fuyu A than in Natsu 4; the quantity of *Cm-HMGR* mRNA in the hybrid was intermediate between that of Fuyu A and Natsu 4 throughout fruit development.

We assayed HMGR activity during fruit development in the three melon genotypes (Fig. 5C). For all three genotypes, HMGR activity increased rapidly during the first week after pollination, then decreased until 21 days after pollination, by which time the activity was undetectable. HMGR activity in the fruit of Fuyu A was higher than that in Natsu 4, whereas that in the hybrid was intermediate between Fuyu A and Natsu 4 throughout fruit development.

Discussion

After cloning the cDNA from the developing fruit of the melon *Cucumis melo* L. *reticulutus*, we characterized *Cm-HMGR* through complementation of the *HMGR*-deficient yeast strain JRY2394 and analyzed transcription levels and HMGR activity during fruit development. We obtained *Cm-HMGR* by chance while screening a cDNA library with a fragment of the gene

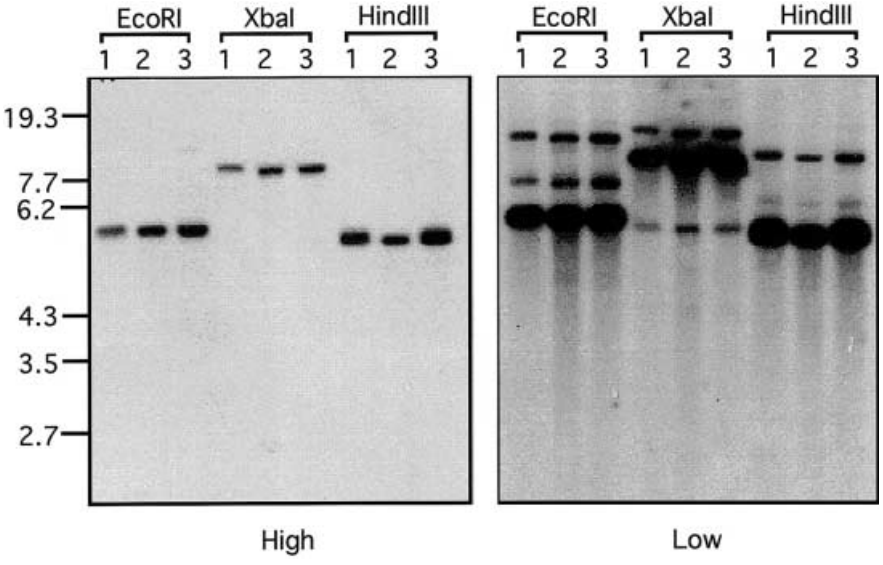

Fig. 4 Southern analysis of melon genomic DNA probed with the 1.25-kb *Cm-HMGR* cDNA fragment encoding the catalytic domains. Digested genomic DNA was electrophoresed through a 0.8% agarose gel and transferred to a nylon membrane. The filter was first hybridized overnight at low stringency (60 °C, Low) and exposed to X-ray film for 4 days. The membrane was then stripped, reprobed overnight at high stringency (65 °C, High), and exposed to film for 2 days. Molecular size markers are indicated on the left. Lanes 1, Fuyu A; 2, Fuyu A × Natsu 4 hybrid; 3, Natsu 4

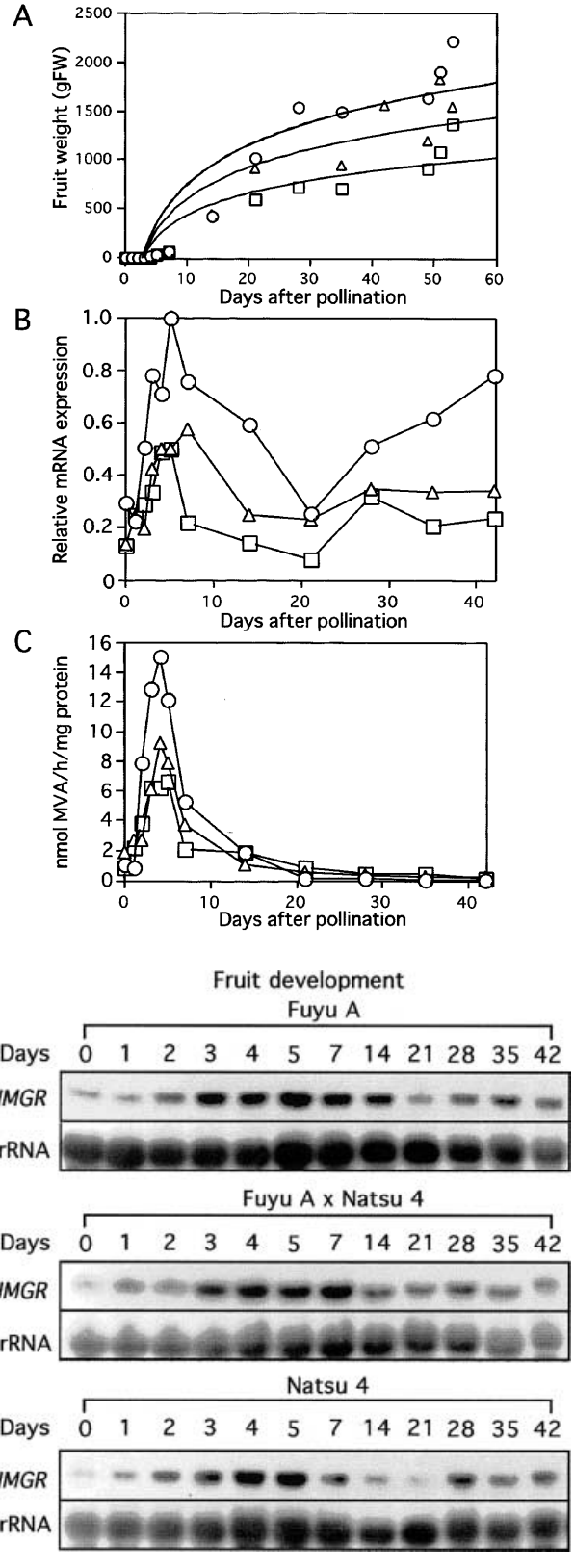

Fig. 5A–D Comparison of fruit growth, HMGR activity, and transcription of *Cm-HMGR* in the melon cultivars Fuyu A and Natsu 4 and the hybrid Fuyu A × Natus 4. **A** Fruit growth after pollination. **B** Relative levels of *Cm-HMGR* mRNA transcripts after pollination. Results are expressed as a percentage of rRNA transcripts. **C** HMGR activity in fruit after pollination. **D** Northern analysis of *Cm-HMGR*. Total RNA was isolated from fruits of each cultivar at 0, 1, 2, 3, 4, 5, 7, 14, 21, 28, 35 and 42 days after pollination. Northern analysis was performed using 10 μg of total RNA per lane and the sample was first probed with the 1.25-kb *Sal*I-*Bgl*II fragment encoding the catalytic domain of Cm-HMGR. After hybridization the membrane was washed and exposed to film. The probe was then removed and the sample was reprobed with an rRNA probe to control for loading

for the melon ethylene receptor, *Cm-ERS1* (Sato-Nara et al. 1999). The degree of homology between *Cm-HMGR* and *Cm-ERS1* is low. However, the high frequency of *Cm-HMGR* transcripts in the cDNA library, which was prepared from fruit tissue, may account for our retrieval of the *Cm-HMGR* cDNA during the screen.

Two peaks of *Cm-HMGR* mRNA accumulation occurred during melon fruit development. The first peak is observed during early fruit development, the other at ripening. *HMGR* mRNA accumulation follows this same pattern during tomato fruit development (Narita and Gruissem 1989; Park et al. 1992), but these peaks are due to differential transcription of two *HMGR* genes, *HMG1* (transcripts of which peaked early during fruit development) and *HMG2* (which is responsible for the ripening-associated peak). The phylogenetic tree of plant HMGRs (Fig. 2) shows that Cm-HMGR and tomato HMG2 belong to different groups; therefore, Cm-HMGR may be a homolog of tomato HMG1. However, characterization of the promoter regions of tomato *HMG1* and *HMG2* (Draselia et al. 1996; Jelesko et al. 1999) suggests that *HMG2* is a gene that evolved late for a specialized function in tomato, rather than playing a role in cell division (Draselia et al. 1996). These results suggest that during fruit development, the transcription of *Cm-HMGR*, the only *HMGR* gene in melon, is regulated similarly to that of the two genes, *HMG1* and *HMG2*, in tomato. Comparison of the promoter region of *Cm-HMGR* with those of tomato *HMG1* and *HMG2* will perhaps provide new insights into the evolution of *HMGR* genes in plants.

Although *Cm-HMGR* mRNA transcripts were expressed during early fruit development and ripening, HMGR activity was associated only with fruit development. Several studies have reported increased HMGR activity which reflect increases in the abundance of HMGR mRNA (Yang et al. 1991; Chye et al. 1992). One possible explanation for our observation is translational regulation of Cm-HMGR during ripening; confirmation of this hypothesis will require a specific antibody against Cm-HMGR. Another possible explanation is post-translational regulation that inactivates Cm-HMGR during fruit ripening (assuming that the protein is actually synthesized during this stage). Reversible phosphorylation, calcium, proteolytic degradation, and subcellular compartmentation all regulate HMGR activity in plants (Stermer et al. 1994); further studies will be needed to determine whether one

of these factors inactivates Cm-HMGR during fruit ripening. Interestingly, HMGR activity and *hmg1* transcription in tomato fruit was highest during early fruit development; however, HMGR activity in ripening tomato fruits was low even though the level of *hmg2* transcription was high (Narita and Gruissem 1989). Together, these results suggest that a common mechanism exists and accounts for the inactivation of HMGR during fruit ripening.

How does *Cm-HMGR* relate to melon fruit development? As in most plants, fruit development in melon is divided into three phases (Gillaspy et al. 1993). Phase I involves ovary development, fertilization, and fruit set. During phase II, fruit growth is primarily due to cell division, whereas growth during phase III occurs mainly by cell expansion and is followed by ripening. We found that *Cm-HMGR* transcription and HMGR activity were markedly increased after melon fruit set, which is coincident with phase II of fruit development. A similar pattern of *HMG1* mRNA accumulation and HMGR activity is observed during the early stage of tomato fruit development (Narita and Gruissem 1989). HMGR catalyzes the conversion of HMG-CoA into mevalonate (MVA), which is the precursor of phytosterols (Gillaspy et al. 1993). Since HMGR is an important control point for the mevalonate pathway in plants (Maurey et al. 1986), the increase in HMGR should result in increasing the supply of phytosterol. In addition, sterols are major components of the eukaryotic cell membrane, and are required for the production of cell membrane, which is a necessary step in cell division. Therefore, *Cm-HMGR* is likely to be involved in cell division during phase II after melon fruit set.

The amount of *Cm-HMGR* mRNA in the fruit of Fuyu A was higher than that in Natsu 4 whereas that in hybrid Fuyu A × Natsu 4 was intermediate between those of the parents; the pattern of accumulation of transcripts seemed to be similar. The level of *Cm-HMGR* mRNA accumulation in the fruit of the three genotypes was associated with fruit size. Interestingly, HMGR activity also correlated with fruit size in melon. In comparison with normal and small-fruit cultivars of avocado, HMGR activities in mesocarp are also associated with cell number and fruit size (Cowan et al. 1997). The amount of cell proliferation during early fruit development determines fruit size in melon (Higashi et al. 1999). Together, these results suggest that by stimulating cell division, *Cm-HMGR* is involved in regulating fruit size in these melon genotypes.

Our results show that *Cm-HMGR* is involved in early events of melon fruit development, most likely cell proliferation after pollination. Transcription levels of *HMG1* in tomato (Narita and Gruissem 1989) and physiological analysis of avocado by using a competitive inhibitor of HMGR (Cowan et al. 1997) point to the importance of HMGR for fruit development in these plants. Further understanding of the role of *Cm-HMGR* in melon fruit development requires direct evidence of the functional connection between cell division and HMGR activity. In addition, analysis of transgenic fruit that expresses Cm-HMGR will provide useful information regarding the association between this protein and fruit development in melon.

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