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3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Activity in the Endosperm of Maize *vivipary* Mutants[1]

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During seed maturation the levels of the rate-limiting enzyme of isoprenoid biosynthesis, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) decrease while the levels of the isoprenoid compound abscisic acid (ABA) increase. In the present study, we demonstrate that HMGR specific activity is inversely correlated with endogenous ABA levels in *Zea mays* endosperm during seed development. HMGR specific activity and ABA levels were measured in the endosperm of the maize vivipary mutants *vp2*, *vp5*, and *vp7*, which are defective in ABA biosynthesis, and *vp1*, which is defective in an ABA response element. Reduced ABA levels were observed in the endosperm of *vp2*, *vp5*, and *vp7*, whereas HMGR activity levels were higher compared with wild-type sibling endosperm activity. HMGR activities were increased by as much as 37% (*vp2*), 45% (*vp5*), and 58% (*vp7*) in the mutants. Endosperm HMGR activity in the *vp1* mutant was also increased (41%) relative to wild-type siblings, even though *vp1* does not have reduced ABA levels. In addition, exogenous ABA inhibits HMGR activity 34 to 50% in maize roots. These results suggest that HMGR activity levels during seed development are regulated via a *Vp1*-dependent signal transduction pathway that is affected by the reduced ABA content of *vp2*, *vp5*, and *vp7* endosperm.

The isoprenoid biosynthetic pathway produces a number of compounds in plants that are essential for growth, development, photosynthesis, and disease resistance (Bach, 1987; Bach et al., 1990). A variety of isoprenoids appear to be required for seed development (Bewley and Marcus, 1990; Belefont and Fong, 1991), but little is known about the specific isoprenoid requirements of seeds or how their biosynthesis is regulated (Moore and Oishi, 1993). During seed development, sterols are thought to be the major isoprenoid compound required for both rapid cell divisions and extensive membrane biosynthesis (Randolph, 1936; Kowels and Phillips, 1985; Haughan et al., 1988; Horbowitz and Obenhorf, 1992). In seeds of *Zea mays* (maize) an additional isoprenoid, ABA, is required (Robichaud et al., 1980; Koornneef et al., 1989). There are two sources of ABA in the developing seed: ABA synthesized by the maternal tissue and imported via the phloem (Karssen et al., 1983) and ABA synthesized by the zygotic tissue of the seed (Karssen et al., 1983; Jones and Brenner, 1987; Koornneef et al., 1989; Belefont and Fong, 1991). ABA produced by the zygotic tissue in maize seeds maintains or controls aspects of maturation, dormancy, and water relations (Robichaud et al., 1980; Jones and Brenner, 1987; Neill et al., 1987; Gage et al., 1989; Belefont and Fong, 1991).

The enzyme HMGR is a key enzyme controlling the isoprenoid pathway in both plants and animals (Gray, 1987; Bach et al., 1990; Goldstein and Brown, 1990; Gondet et al., 1992). Plant microsomal HMGR has been shown to be regulated by light (Brooker and Russell, 1979; Ji et al., 1992; Moore and Oishi, 1993), phytohormones (Brooker and Russell, 1979; Russell and Davidson, 1982), wounding, fungal infection, and fungal elicitors (Stermer and Bostock, 1987). Results from our studies of HMGR activity during seed development suggest that this enzyme and its regulation are important during seed development. We have shown that HMGR activity is regulated during maize seed development, both temporally and spatially in the embryo and endosperm (Moore and Oishi, 1993). Additionally, our studies suggest that there is an inverse correlation between ABA levels and HMGR activity (Moore and Oishi, 1993). Examination of WT seeds revealed that endosperm HMGR activity remains low and relatively constant during the increase of zygotic ABA, from 14 to 30 DAP. Finally, the hypothesis that ABA may regulate HMGR activity was initially suggested by Russell and Davidson (1982), who demonstrated that exogenous ABA decreases HMGR activity in apical pea buds.

Maize represents an ideal system in which to study the effect of ABA on HMGR activity during seed development. Seed development in maize is well defined both morphologically and genetically (Randolph, 1936; Kiesselbach, 1949; Kowels and Phillips, 1985; Neuffer et al., 1986). In addition, a set of maize ABA-deficient mutants (*vivipary* mutants) exists that exhibit altered seed development and are useful for studying the role of ABA in the regulation of isoprenoid biosynthesis (Robertson, 1955; Robichaud et al., 1980; Neill et al., 1986).

In the present study, we used the maize *vivipary* mutants to determine in vivo whether endogenous levels of ABA affect HMGR activity during seed development. We report that HMGR specific activity is inversely correlated with endogenous ABA levels in maize endosperm during seed de-

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Abbreviations: DAP, days after pollination; HMGR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; MVA, mevalonate; WT, wild type.

velopment and conclude that ABA may regulate HMGR activity via a *Vp*1-dependent signal transduction pathway.

MATERIALS AND METHODS

Plant Material

Seeds of *Zea mays* lines (*Vp*1/*vp*1), (*Vp*5/*vp*5), (*Vp*2/*vp*2), and (*Vp*7/*vp*7) were obtained from the Maize Stock Center (University of Illinois, Urbana, IL). Heterozygous lines of *vp*2, *vp*5, and *vp*7 were outcrossed to the inbred line FunkF two or three times to place them into a common genetic background ensuring that the temporal development of the seed would be identical among the different mutants. The homozygous mutant *vp*2, *vp*5, and *vp*7 lines synthesize reduced amounts of zygotic ABA during seed development, resulting in precocious germination (Robertson, 1955; Robichaud et al., 1980; Neill et al., 1986). The line *Vp*1/*vp*1 was maintained in a W22 inbred background; *vp*1 seeds contain normal levels of ABA and have reduced sensitivity to exogenous ABA (Robichaud et al., 1980). These recessive mutants are lethal as homozygous plants; therefore, the mutations are maintained as heterozygotes (+/*vp*). To obtain *vp* kernels, heterozygous plants were crossed to one another resulting in kernels segregating 1:3 *vp* to WT (+/−). To prevent any potential artifacts due to diurnal variation in HMGR activity (Wititsuwannakul, 1986), all seeds were harvested between 10 and 11 AM.

The *vp*2, *vp*5, and *vp*7 mutants accumulate specific intermediate carotenoid compounds due to lesions in the biosynthetic pathway (Robertson, 1955; Fig. 1) resulting in the interruption of ABA biosynthesis (Robichaud et al., 1980). Homozygous *vp* kernels have reduced amounts of carotenoids, and the resulting pale yellow (*vp*2), white (*vp*5), or pink (*vp*7) endosperms are easily distinguished from the WT yellow endosperm as early as 14 DAP. Since precocious germination of the embryo is not visible until late maturation (18–20 DAP), mutant *vp* kernels were identified at earlier stages based on endosperm color. The *vp*1 mutant has WT levels of ABA and carotenoids but has greatly reduced amounts of a transcription factor involved in ABA-inducible gene expression (McCarty et al., 1991). This mutation has pleiotropic effects, including decreased anthocyanin biosynthesis in the aleurone layer of the kernel (Eyster, 1931). In the W22 background, *vp*1 seeds have a colorless aleurone layer that can be easily distinguished from the purple aleurone of WT seeds at 20 DAP.

Dry seeds from the inbred line FunkF (backcrossed for four generations) were used for the germination and exogenous ABA studies. Whole seeds were surface sterilized in a 10% (v/v) solution of commercial bleach. For the germination study, seeds were rinsed in tap water, rolled in germination paper, and allowed to imbibe in H_2O for 0 to 72 h in total darkness at 28°C. Seeds were de-embryonated, the pericarps were removed, and microsomal fractions were isolated from the remaining endosperm tissue. To study the effect of exogenous ABA on HMGR activity, seeds were surface sterilized and germinated in rolled germination paper for 48 h. Seedlings were then transferred to beakers containing either H_2O or 50 μM (±)-ABA. Seedlings were kept aerated for 3, 6, 12, or 24 h in total darkness. Microsomal fractions were isolated from root tissue at 3, 6, 12, and 24 h.

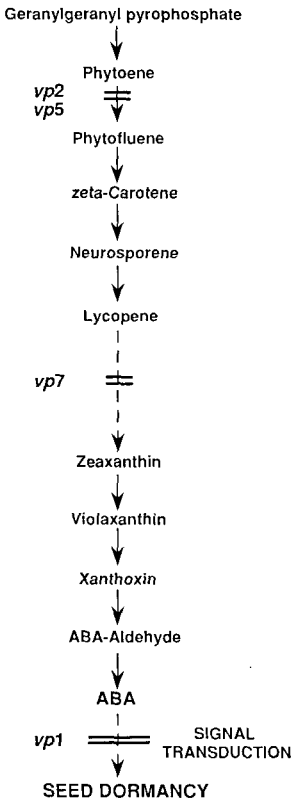

Figure 1. General scheme of carotenoid and ABA biosynthesis showing positions of the *vp*2, *vp*5, *vp*7, and *vp*1 mutations (Moore and Smith, 1985; Neill et al., 1986; Bramley and MacKenzie, 1988).

Microsomal Isolation and Measurement of HMGR Specific Activity

Field-grown ears of maize were harvested between 10 and 11 AM, the pericarp was removed, and kernels were de-embryonated. On the day of harvest, microsomal fractions were isolated from endosperm and cytosolic HMGR specific activity was measured as described by Moore and Oishi (1993). Microsomal protein was measured according to the method of Ghosh et al. (1988) using BSA as a standard. Multiple microsomal fractions were isolated and assayed for each time point from three independent field experiments (spring 1992, fall 1992, spring 1993). Data are presented as means ± SE of two to six independent assays. SE averaged <12% of the mean.

Analysis of ABA Levels

ABA levels were determined by tracer competition immunoassays (Walker-Simmons, 1987). All ABA procedures were performed under green light. For ABA extractions, the endosperm from 5 to 10 seeds was frozen in liquid nitrogen, lyophilized, and ground to a fine powder. ABA was extracted according to the method of Oishi and Bewley (1990), with

modifications. The powder was extracted in 1.0 mL of cold methanol containing butylated hydroxytoluene (10 $\mu g\ mL^{-1}$) and citric acid monohydrate (0.5 mg mL^{-1}) at 4°C for 30 to 60 min (Oishi and Bewley, 1990). The extraction buffer also contained 7000 dpm of [2-^{14}C]ABA (Amersham, gift of Dr. Hans Bohnert, University of Arizona, Tucson, AZ). The extract was centrifuged at 48,000*g* for 5 min, the methanol was decanted, and the tissue was rinsed with 500 μL of the extraction buffer. The methanol fractions were combined, vacuum dried, and resuspended in 200 to 400 μL of Tris-buffered saline (pH 7.5; 25 mM Tris-HCl, 0.1 M NaCl, 1.0 mM $MgCl_2$). Samples were stored at −80°C until assayed. ABA was determined using the Phytodetek (Idetek, San Bruno, CA) monoclonal antibody ELISA system using (±)-ABA as a standard. All data were corrected for losses during purification by measuring recovery of [^{14}C]ABA added to the samples before purification. Recovery ranged from 58 to 100% with an average of 90%.

Chemicals

All reagents were analytical grade. [^{14}C]HMG-CoA and [^{3}H]MVA were purchased from New England Nuclear Research Products. Silica gel plates were purchased from Alltech Associates (Deerfield, IL). Monoclonal antibody to ABA was purchased from Idetek. All other reagents were purchased from Sigma.

RESULTS

Expression of HMGR Activity in the Endosperm of *vivipary* Mutants Blocked in the Biosynthesis of ABA

We examined HMGR specific activity in the endosperm of three nonallelic vivipary mutants, *vp2*, *vp5*, and *vp7*. To study seeds with an identical genetic background, cytosolic HMGR activity was measured in mutant (*vp*) and WT endosperm that had been isolated from the same ear. Figure 2 illustrates HMGR specific activity levels from both *vp* and WT endosperms isolated at selected DAP, expressed in units of nmol of MVA h^{-1} mg^{-1} of protein. In the *vp* endosperm of all three mutants, HMGR activity was greater relative to WT HMGR activity (Fig. 2). This difference was observed particularly during maturation (approximately 14–20 DAP; Randolph, 1936; Kiesselbach, 1949; Wilson, 1983). During maturation, HMGR activity in *vp* endosperm was greater by 30 to 37% (*vp2*), 23 to 45% (*vp5*), and 16 to 58% (*vp7*) compared with the activity in WT endosperm. Although the magnitude of HMGR activity increased in all three of the *vp* endosperm during maturation, the relative temporal HMGR activity profiles were similar in both WT and mutant and were similar to the temporal profiles observed in WT FunkF endosperm during seed development (Moore and Oishi, 1993; K.B. Moore and K.K. Oishi, unpublished observations). Variability in the levels of HMGR activity among the different mutant lines was observed, but the trends were similar in all experiments, with *vp* endosperm showing increased activity relative to WT. This observed difference in HMGR activity of *vp* endosperm does not appear to be due to differences in age of the seed or increased water content of the mutant tissue, since both WT and *vp* endosperm have similar fresh and dry

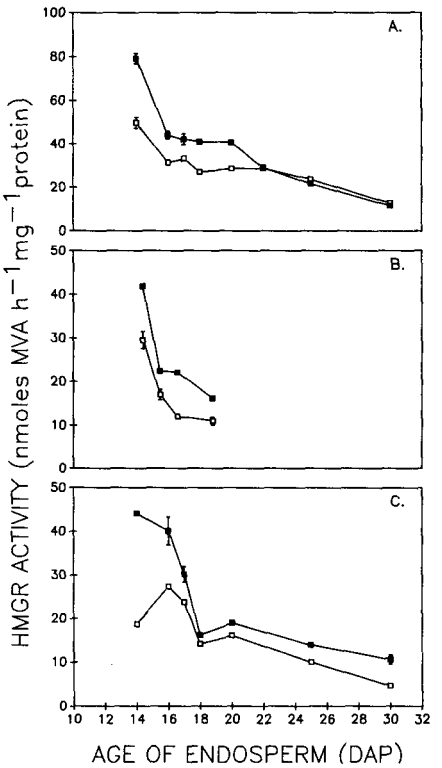

Figure 2. Microsomal HMGR specific activity in (*vp*/*vp*/*vp*) (■) or WT (+/+/−) (□) *vp2* (A), *vp5* (B), and *vp7* (C) endosperm during seed development. Enzyme activity for both mutant and WT endosperm was determined as described in "Materials and Methods." Each value represents the mean ± SE of three to six determinations from a representative experiment. SE ranged from 0.05 to 10% with an average of 7%.

weights from 14 to 20 DAP (Table I). By 25 to 30 DAP, HMGR activity decreased in both *vp* and WT endosperm in all three mutants (Fig. 2); this pattern is similar to the WT FunkF endosperm activity profile (Moore and Oishi, 1993). These experimental results demonstrating the negative correlation between ABA and HMGR activity have been confirmed in a third independent field test using *vp2*, *vp5*, and *vp7* mutants as well as additional *vp* mutants, and we have also determined that HMGR activity is decreased in *vp* mutant embryos during seed development (data not shown).

The maize mutants *vp2*, *vp5*, and *vp7* are defective in the biosynthesis of carotenoids and ABA and thus accumulate lower amounts of zygotic ABA (Brenner et al., 1977; Neill et al., 1986). To determine levels of ABA in the endosperm of the *vp2*, *vp5*, and *vp7* alleles in the FunkF background, ABA levels were measured. Mutant endosperms accumulate 40 to 80% of the ABA levels found in WT endosperm (Table II). Since both mutant and WT seeds are present on the same ear, *vp* and WT endosperm presumably receive the same source and amount of maternal ABA. Therefore, the decrease in ABA content of the *vp* endosperm is most likely due to a decrease in zygotically produced ABA.

Viviparous germination of *vp* embryos is typically visible from 17 to 20 DAP, during the time in which differences are

Table I. *Fresh and dry weights of whole endosperm during seed development in WT and vivipary mutant seeds*

Homozygous mutant (*vp*) or WT seeds (+/−) were harvested at various times during seed development. Seeds were de-embryonated and the fresh weight was determined. Endosperm were quick frozen in liquid nitrogen and vacuum dessicated to obtain dry weights. Values are from a representative experiment.

Age of Seed	Fresh Wt	Dry Wt	Fresh Wt	Dry Wt
DAP	*g*	*g*	*g*	*g*
	WT		*vp2*	
14	0.086	0.015	0.085	0.013
16	0.109	0.023	0.097	0.020
17	0.149	0.045	0.149	0.041
18	0.149	0.046	0.141	0.044
20	0.170	0.069	0.169	0.058
25	0.258	0.126	0.190	0.101
30	0.275	0.142	0.193	0.107
	WT		*vp5*	
14	0.109	0.024	0.110	0.023
15	0.118	0.026	0.122	0.025
16	0.143	0.039	0.137	0.038
18	0.152	0.055	0.138	0.051
	WT		*vp7*	
14	0.135	0.028	0.124	0.024
16	0.149	0.040	0.146	0.038
17	0.159	0.049	0.156	0.042
18	0.169	0.049	0.152	0.042
20	0.173	0.073	0.163	0.070
25	0.167	0.094	0.139	0.082

Table II. *Endogenous ABA levels in WT and vp endosperm*

Relative levels of active (*S*)-ABA were determined for each developmental time point using trace competition ELISA (Idetek). ABA was extracted from five mutant (*vp/vp/vp*) or WT (+/+/−) endosperm for each developmental time point. The mean and SE for three to six repetitions are shown.

Age of Seed	Amount of ABA	
DAP	*pmol g⁻¹ dry wt*	
	WT	*vp2*
14	33.0 ± 0.0	26.5 ± 1.2
16	33.6 ± 1.9	19.2 ± 3.2
17	15.1 ± 0.4	9.5 ± 0.6
18	17.4 ± 0.5	10.2 ± 0.7
20	14.6 ± 1.5	6.6 ± 0.3
25	12.2 ± 0.9	5.9 ± 0.8
30	5.4 ± 0.7	3.2 ± 0.4
	WT	*vp5*
14	26.8 ± 2.2	18.4 ± 0.8
15	20.7 ± 0.1	14.4 ± 2.1
16	19.2 ± 0.6	12.4 ± 1.1
18	10.3 ± 0.5	7.4 ± 0.2
	WT	*vp1*
22	8.8 ± 0.8	8.1 ± 1.0
25	11.2 ± 1.5	11.6 ± 1.2
27	13.6 ± 2.9	13.2 ± 0.1
31	18.6 ± 2.7	18.5 ± 0.9

observed in endosperm HMGR activity (Fig. 2). During germination, the endosperm also changes, undergoing degradation and absorption by the embryo as its storage reserves are mobilized for the seedling (Bewley and Black, 1985). Thus, it is possible that the increase in HMGR activity of the *vp* endosperm is due to the changing developmental stage of the endosperm or is an effect of embryo germination. To rule out the possibility that the changes in endosperm HMGR activity are due to an effect of germination, we measured HMGR specific activity in the endosperm of WT germinating seedlings. These measurements indicate that HMGR activity remains at basal levels for the first 72 h of imbibition/germination, ranging from 1.2 ± 0.1 to 2.7 ± 0.2 units of activity (Table III). These levels of HMGR activity are substantially lower than the levels in the *vp* endosperm (Fig. 2), and are 10-fold lower than levels of HMGR activity in endosperm during WT seed development (Moore and Oishi, 1993).

Expression of HMGR Specific Activity in *vp1* Endosperm

Unlike the other *vp* mutants, *vp1* mutant endosperm has WT levels of both carotenoids (yellow color) and ABA (Neill et al., 1986). We confirmed that the *vp* endosperm of the *vp1* allele used in this study contains the same levels of ABA as the WT endosperm (Table II). Since WT and mutant seeds could not be distinguished until approximately 20 DAP, measurements of *vp1* HMGR activity were made somewhat later during seed development. The *vp1* mutant also is maintained in a different genetic background (W22), which develops more slowly in Arizona than the FunkF inbred lines of carotenoid biosynthetic *vp* mutants (K.B. Moore and K.K. Oishi, unpublished observations). Therefore, a direct comparison of levels of HMGR activity at specific DAP cannot be made between the *vp1* and other *vp* mutants. Figure 3 illustrates HMGR specific activity for *vp1* and WT endosperm during seed development. The pattern of HMGR activity in the *vp1* mutant is similar to the patterns observed in *vp2*,

Table III. *HMGR specific activity in endosperm of imbibing and germinating embryos*

Enzyme activity of de-embryonated endosperm from FunkF seeds after 24, 36, 48, 60, and 72 h of imbibition/germination. Values are from a representative experiment, and each data point is the mean ± SE of two to four replications. SE ranged from 0.05 to 15%.

Time of Imbibition	Units of Specific Activity
h	*nmol MVA h⁻¹ mg⁻¹ protein*
0	2.6 ± 0.0
24	2.1 ± 0.2
36	2.7 ± 0.2
48	2.5 ± 0.1
60	1.2 ± 0.1
72	1.3 ± 0.2

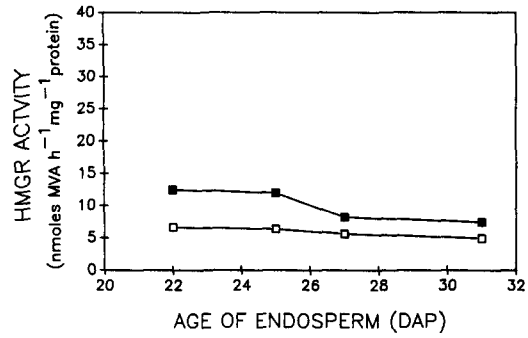

Figure 3. Microsomal HMGR specific activity in (*vp*/*vp*/*vp*) (■) or WT (+/+/−) (□) *vp*1 endosperm during seed development. HMGR activity was measured as described previously. Each HMGR value is the mean ± SE of two to four determinations from a representative experiment. SE ranged from 0.1 to 5% with an average of 3%.

*vp*5, and *vp*7 mutants (Figs. 2 and 3). HMGR activity is greater in the *vp*1 endosperm by 34 to 41% relative to WT (Fig. 3).

Effect of Exogenous ABA on HMGR Activity

Since it is difficult to culture endosperm, young maize seedlings were grown in the presence of 50 μM (±)-ABA to determine whether maize cytosolic HMGR activity is affected by exogenous ABA. The results from analysis of root HMGR activity show lower activity levels in the roots of seedlings treated with ABA for 3, 6, 12, and 24 h compared with controls (Table IV). HMGR activity in the ABA-treated seedlings ranged from 50 to 68% of control activity, and the lower HMGR activity was correlated with a decrease in root growth at 12 and 24 h. However, a difference in HMGR activity between ABA-treated and untreated seedlings was observed after 3 h of treatment, whereas a visible effect on growth was not observed until 12 h.

DISCUSSION

Analysis of cytosolic HMGR specific activity in three *vp* mutants demonstrates that HMGR activity is greater in *vp* compared with WT endosperm. Since these mutants accumulate lower amounts of ABA, these results suggest that ABA is involved in modulating HMGR activity. This difference in HMGR activity is particularly apparent during early to mid-maturation (14–20 DAP) and is not due to changes in the developmental state of the endosperm caused by germination (Table III). The *vp*2 and *vp*7 mutations block carotenoid biosynthesis at two different steps in the carotenoid/ABA biosynthetic pathway, and each accumulates a specific carotenoid intermediate compound (Fig. 1). Thus, it appears that the observed increase in HMGR activity is the result of a deficiency in accumulation of zygotic ABA in the *vp* endosperm rather than an affect of either phytoene (*vp*2, *vp*5) or lycopene (*vp*7) accumulation. These data indicate that genetic alterations that decrease endogenous ABA levels correlate negatively with HMGR activity, and we hypothesize that high levels of ABA may suppress HMGR activity in vivo. In particular, this potential feedback inhibition has been observed during a specific developmental process in which ABA may play a role (Wilson et al., 1973; Myers et al., 1990; Oishi and Bewley, 1990; Ober et al., 1991). The mechanism of this inhibition in plants is unknown, particularly since this is one of the few demonstrations in plants of feedback inhibition of HMGR activity by an isoprenoid end product.

In all three *vp* mutants analyzed, the basic temporal pattern of HMGR activity was maintained in the *vp* endosperm, with the magnitude of the HMGR activity greater in the mutants (Fig. 2). This is the first genetic evidence implicating ABA, a product of the isoprenoid biosynthetic pathway in the regulation of HMGR activity during seed development. Since the temporal HMGR activity pattern is not altered in the *vp* tissue, this may be an indication that ABA plays a role in the level of induction of HMGR activity during seed development but that it may not function in determining the overall developmental pattern of activity. Instead, the temporal regulation of HMGR activity appears to be tightly controlled by the developmental state of the tissue (Moore and Oishi, 1993; Fig. 2).

Consistent with the hypothesis that ABA is a negative regulator of HMGR activity, we have also shown that exogenous ABA inhibits cytosolic HMGR activity in maize roots (Table IV). It is interesting that this inhibition is observed well before an effect on root growth is visible. We have also shown that HMGR activity is decreased in maize and cotton roots under conditions of salt stress, conditions under which ABA levels have been shown to increase (Kefu et al., 1991; data not shown). This study provides additional evidence that endogenous ABA may function in feedback inhibition of plant HMGR.

Plant isoprenoid synthesis is a highly compartmentalized process that occurs in the cytosol, chloroplast, and mitochondria. It has been hypothesized that plant HMGRs may exist in specialized isozyme forms located within these different cell compartments. The existence of a plastid HMGR is controversial and unresolved at present (Gray, 1987; Bach et al., 1990); however, the available evidence in maize shows that HMGR activity is associated mainly with microsomal rather than plastid membranes (Ji et al., 1992; Moore and Oishi,

Table IV. *HMGR specific activity in roots of seedlings treated with exogenous ABA*

Enzyme activity from roots of 20 to 25 FunkF seedlings after 6, 12, and 24 h of treatment with 50 μM (±)-ABA. The average root length ± SE is given for each time. Values are from a representative experiment, and each data point is the mean ± SE of four replications. SE ranged from 0.01 to 0.2%.

Time of Treatment	Average Root Length		Units of Specific Activity	
	Control	ABA-treated	Control	ABA-treated
h	*mm*		*nmol MVA h^{-1} mg^{-1} protein*	
3	28.7 ± 11.2	28.3 ± 11.6	31.3 ± 0.1	19.2 ± 0.4
6	34.3 ± 10.1	28.6 ± 8.0	23.8 ± 0.8	16.3 ± 1.2
12	25.0 ± 9.2	20.0 ± 8.0	46.0 ± 0.1	23.0 ± 0.3
24	46.8 ± 13.7	36.7 ± 10.5	46.5 ± 0.9	25.2 ± 0.7

1993). Thus, it seems unlikely that plastid isoform would contribute significantly to the HMGR activity during seed development. Furthermore, the pathway and location of ABA synthesis in the seed are at present unresolved. Two pathways of ABA synthesis have been proposed, and both are branches of the isoprenoid pathway with MVA as a specific precursor; thus, feedback regulation of HMGR by ABA is feasible in either pathway. It is unlikely that ABA itself has a direct allosteric role in regulating HMGR expression in the endosperm, since the addition of ABA to HMGR assays in vitro has no effect on HMGR activity (Russell and Davidson, 1982). More likely, ABA has an indirect regulatory effect, modulating the levels of cytosolic HMGR activity via an established ABA signal transduction pathway that functions during seed development.

To determine whether HMGR activity is regulated during seed development via a *Vp*1-dependent signal transduction pathway, we examined HMGR activity in the endosperm of *vp*1 mutants. Our results show that HMGR activity is increased in the *vp*1 endosperm tissue. Since *vp*1 seeds contain WT levels of carotenoids and ABA, these data further indicate that it is not the presence or absence of carotenoids or ABA that is responsible for the observed increase of HMGR activity in the *vp* mutants. Rather, since the *vp*1 mutants are not blocked in the synthesis of ABA but are defective in some element of the ABA response, these data suggest that ABA per se does not regulate HMGR expression during seed development but that HMGR activity is regulated by ABA in a *Vp*1-dependent manner during seed development.

The product of the *Vp*1 gene has been shown to be a transcription factor involved in the complex ABA signal transduction during seed development (McCarty et al., 1989, 1991; Pla et al., 1991). It is possible that ABA regulates HMGR activity during seed development transcriptionally via *Vp*1, but currently it is not clear whether the VP1 protein is directly or indirectly involved in the regulation of HMGR expression. VP1 protein has been shown to both induce and repress the expression of genes (Kriz et al., 1990; McCarty et al., 1991; Hoecker et al., 1993); our study suggests that VP1 could function normally to suppress HMGR activity during seed development, perhaps through repression of one or more HMGR genes.

Although our data are consistent with the hypothesis that ABA regulates HMGR activity during seed development by altering HMGR gene expression, the present study does not rule out the possibility that changes in ABA levels may cause pleiotropic changes in seed morphology and physiology that are reflected in changes in HMGR specific activity. There are several possibilities as to why ABA might regulate HMGR activity. ABA may be regulating its own synthesis during seed development via HMGR activity levels, similar to the role that cholesterol plays in the multivalent feedback regulation of mammalian HMGR activity, although ABA may act more indirectly. Alternatively, ABA may be involved in limiting the growth (cell number and/or size) of the endosperm tissue by down regulating MVA concentrations and thus limiting the pools of sterol precursors necessary for cell division or membrane expansion. We are currently investigating the mechanism(s) by which ABA regulates HMGR activity during seed development.

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