The *yellow variegated* Mutant of *Arabidopsis* Is Plastid Autonomous and Delayed in Chloroplast Biogenesis

M. Chen, M. Jensen, and S. Rodermel

The *yellow variegated* mutant of *Arabidopsis thaliana* is characterized by bright-yellow true leaves that turn green- and white-sectored as leaf development proceeds. Variegation is due to the action of a nuclear recessive gene. Whereas cells in the green sectors contain morphologically normal chloroplasts, cells in the yellow and white sectors are heteroplastidic and contain plastids with rudimentary lamellar structures, as well as some normal-appearing chloroplasts. This indicates that plastids in *yellow variegated* are affected differently by the nuclear mutation (the mutant is "plastid autonomous"). Genetic analyses have revealed that *yellow variegated* is an allele of the *var*2 locus, and that defective plastids are not maternally inherited. The traits of plastid autonomy and lack of maternal inheritance of the plastid defect set *var*2 apart from other nuclear gene-induced variegations and define a novel class of variegation mutant. The primary lesion in *var*2 probably does not involve a blockage in the pathways of pigment biosynthesis. Under high temperatures or low light conditions, plant growth is retarded and mutant plants are nearly all-green. Considered together, our data suggest that *var*2 is delayed in chloroplast biogenesis. We suggest that the stochastic pattern of variegation in the mutant may be due to an interplay of factors that regulate *var*2 gene expression and factors that mediate rates of cell and plastid division. Plastids with a critical threshold of the partially functional *var*2 protein are green, while plastids containing less than the threshold of *var*2 activity are white.

Chloroplast biogenesis is coordinated with leaf development and involves a complex interplay of exogenous and endogenous factors (reviewed in Mullet 1988; Susek and Chory 1992; Taylor 1989). The signal transduction pathways that regulate chloroplast development, as well as the mechanisms that integrate organelle and nuclear gene expression during this process, are poorly understood. In higher plants, a molecular genetic dissection of nuclear gene-induced variegation mutants is a powerful approach to identify nuclear signals that affect plastid development (e.g., Coe et al. 1988; Giuliano et al. 1993; Gu et al. 1993; Han et al. 1992; Hess et al. 1992, 1994; Martínez-Zapater 1993; Martínez-Zapater et al. 1992; Meehan et al. 1996; Newton and Coe 1986; Rédei 1963, 1967, 1973; Rhoades 1943; Roussell et al. 1991; Walbot and Coe 1979; Wetzel et al. 1994). These mutants have leaves that contain green and white (and/or yellow) sectors. Whereas cells in the green sectors contain morphologically normal chloroplasts, cells in the white sectors generally contain nonpigmented plastids with rudimentary lamellar structures. These observations indicate that the nuclear gene product defined by the mutation is required for normal chloroplast differentiation.

We have been studying the *immutans* (*im*) variegation mutant of *Arabidopsis* (Meehan et al. 1996; Rédei 1963, 1967; Wetzel et al. 1994; Wetzel and Rodermel 1998). Like many other nuclear gene-induced variegations, *im* gives rise to defective plastids in homozygous recessive plants. Unlike these mutants, the plastid defect in *im* is not maternally inherited. The somatic instability in *im* is modulated by light and temperature, with white sector formation being promoted by enhanced temperatures, light intensities, and/or red light. Phytoene, a noncolored carotenoid precursor, accumulates in white sectors of the mutant, indicating that *im* affects the activity of phytoene desaturase (PDS), the enzyme that converts phytoene to ζ-carotene. However, *im* is not the structural gene for PDS (Wetzel et al. 1994), nor does it affect PDS mRNA and protein accumulation (Wetzel and Rodermel 1998).

From the Interdepartmental Plant Physiology Program and Department of Botany, Iowa State University, Ames, Iowa. The authors wish to express their appreciation to G. P. Rédei and J. Martínez-Zapater for providing *A. thaliana* seeds, and to Jack Horner and Bruce Wagner of the Bessey Microscopy Facility (Iowa State University) for electron microscopy. This work was supported by grants from the Iowa State Biotechnology Council and DOE (DE-FG02–94ER20147) to S.R.R. This is journal paper no. J-18026 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, project no. 2987, and supported by Hatch Act and State of Iowa funds. Address correspondence to Steven Rodermel, Department of Botany, 353 Bessey Hall, Iowa State University, Ames, IA 50011, or e-mail: rodermel@iastate.ed.

 90:207–214

The *yellow variegated* mutant of *A. thaliana* was isolated by Rédei in the 1950s (personal communication). We first became interested in this mutant because of its superficial resemblance to *im.* In this article we show that *yellow variegated* does not complement *im,* but that it is allelic to the *var*2 locus, first reported by Martínez-Zapater (1993). *var*2 is only one of four nuclear variegation loci that have been described in *A. thaliana*—the others being *im, chloroplast mutator* (Martínez-Zapater et al. 1992; Rédei 1973; Sakamoto et al. 1996), and *var*1 (Martínez-Zapater 1993). Martínez-Zapater (1993) described the gross morphology of mature *var*2 plants and examined the affects of temperature and light on white-sector formation. He also mapped *var*2 to chromosome 2. In this article we confirm and extend the observations of Martínez-Zapater, and also examine aspects of the ultrastructure, biochemistry, and molecular biology of *var*2 to gain insight into the primary molecular lesion in the mutant. We conclude that *var*2 is representative of a novel class of nuclear gene-induced variegation mutant.

Materials and Methods

Plant Material, Growth, and Genetic Analyses

Mutant seed stocks were obtained from Dr. G. P. Rédei (University of Missouri), Dr. Jose Martínez-Zapater (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain), the *Arabidopsis* Biological Resource Center (ABRC), Ohio State University, and the Nottingham *Arabidopsis* Stock Centre. Wild-type and *var*2 plants were maintained in growth chambers at 22°C under continuous illumination (100 μmole/s/m^2) using fluorescent and incandescent lights. In some experiments, plants were grown under a 16 h light, 8 h dark regime. Complementation tests and analyses of maternal inheritance were performed as described in Wetzel et al. (1994).

Electron and Chlorophyll Fluorescence Microscopy

Samples for electron microscopy included cotyledons and first true leaves of wild-type and *var*2 plants. Small leaf punches were obtained from green, yellow, and white sectors, and the samples were fixed, stained, and examined as described in Horner and Wagner (1980). Phase and chlorophyll fluorescence microscopy were carried out as described in Wetzel et al. (1994). Fresh variegated leaves were placed on slides with water and a coverslip for viewing, and the photographed focal plane was in the mesophyll layer of the leaf. The photographs were taken within 30 min of leaf detachment to minimize the affects of cell damage.

Pigment Analyses

HPLC analyses of chlorophyll and carotenoid pigments were carried out as described in Wetzel et al. (1994). For the determination of total chlorophyll *a* and *b* and carotenoids, pigments were extracted in DMSO at 65°C for 30 min and their concentrations were determined spectrophotometrically (Hiscox and Israelstam 1979; Hodgins and van Huystee 1986).

δ-ALA Feeding Experiments

δ-ALA feeding experiments were performed essentially as described in Runge et al. (1995). In brief, *var*2 and wild-type plants were grown in continuous light in soil. After the seedlings had produced their first pair of true leaves, the plants were gently removed from the soil, the roots were rinsed, and the cotyledons were excised. The plants were then placed in a microfuge tube and vacuum infiltrated with 10 mM δ-ALA-KOH (pH 7.0) for 1 min, followed by 18 h in either darkness or light. Pigments were extracted in acetone under a green safelight, and protochlorophyllide concentrations were measured using a fluorometer (excitation 440 nm, emission 630 nm).

Results

Phenotype of *yellow variegated*

The *yellow variegated* mutant of *A. thaliana* was isolated by Dr. G. P. Rédei (University of Missouri) following X-ray mutagenesis of Columbia seeds (personal communication). The mutant is characterized by variegated stems, true leaves (rosette and cauline), sepals, and siliques. Whereas the cotyledons appear normal, the "yellow" comes from the striking appearance of emerging true leaves, which are bright yellow. This feature is illustrated in Figure 1B for a severe allele of *yellow variegated* (described later). Small green islands first appear on the leaves a few days after visible emergence, and as the leaves expand, the yellow color gradually fades out to leave green- and ivory-colored sectors (Figure 1A). The leaves become more green as they expand, but sector boundaries become fixed at full expansion, that is, white sectors do not turn green after full expansion is attained. The paired first leaves are always more variegated than later leaves. Leaves that emerge just prior to bolting are often all green.

Mode of *yellow variegated* Inheritance

Rédei found that *yellow variegated* is expressed only in homozygous recessive plants (personal communication). Complementation analyses using a number of "variegated" mutants from the Ohio State and Nottingham stock centers revealed that *yellow variegated* is allelic to at least four variegation mutations that have been isolated in *A. thaliana.* These include two alleles of the *var*2 locus, *var*2–1 and *var*2–2 (Martínez-Zapater 1993) (Table 1). In keeping with Martínez-Zapater's nomenclature, we designate the new *var*2 alleles as *var*2–3, *var*2–4 (*yellow variegated*) and *var*2–5 (Table 1). The phenotype of *var*2–1 is illustrated in Figure 1A,B.

Plastids are strictly maternally inherited in *A. thaliana* (reviewed in Rédei 1975). To determine whether mutant plastids are maternally inherited in *yellow variegated,* we reciprocally crossed mutant and wild-type plants (Table 2). Variegated reproductive structures were used in the crosses. If *yellow variegated* produced permanently defective plastids (e.g., due to chloroplast gene mutations), then nonpigmented plastids would be transmitted from variegated females to F_1 progeny plants. We observed that all F_1 progeny from these crosses, regardless of parentage of the cytoplasm, had a wild-type phenotype. Hence defective plastids are not maternally inherited in *yellow variegated.* The data in Table 2 also confirm Rédei's initial observation that *yellow variegated* segregates in the F_2 progeny in a 3:1 ratio (wild type : mutant), as expected of a Mendelian recessive mutation.

*var*2 Is Delayed in Pigment Accumulation

The observations in Figure 1A,B suggested to us that *var*2 may be delayed in pigment accumulation during leaf and shoot morphogenesis. Consistent with this hypothesis, Figure 2 shows that chlorophyll concentrations, although somewhat reduced in the mutant cotyledons, are dramatically depressed in emerging (yellow) first leaves. Pigment concentrations subsequently increase such that fully expanded (mature) *var*2 first leaves have nearly normal pigment amounts. This general pattern of pigment accumulation is also true for subsequent leaves. In the wild type, chlorophyll concentrations are reduced in fully expanded versus young, expanding

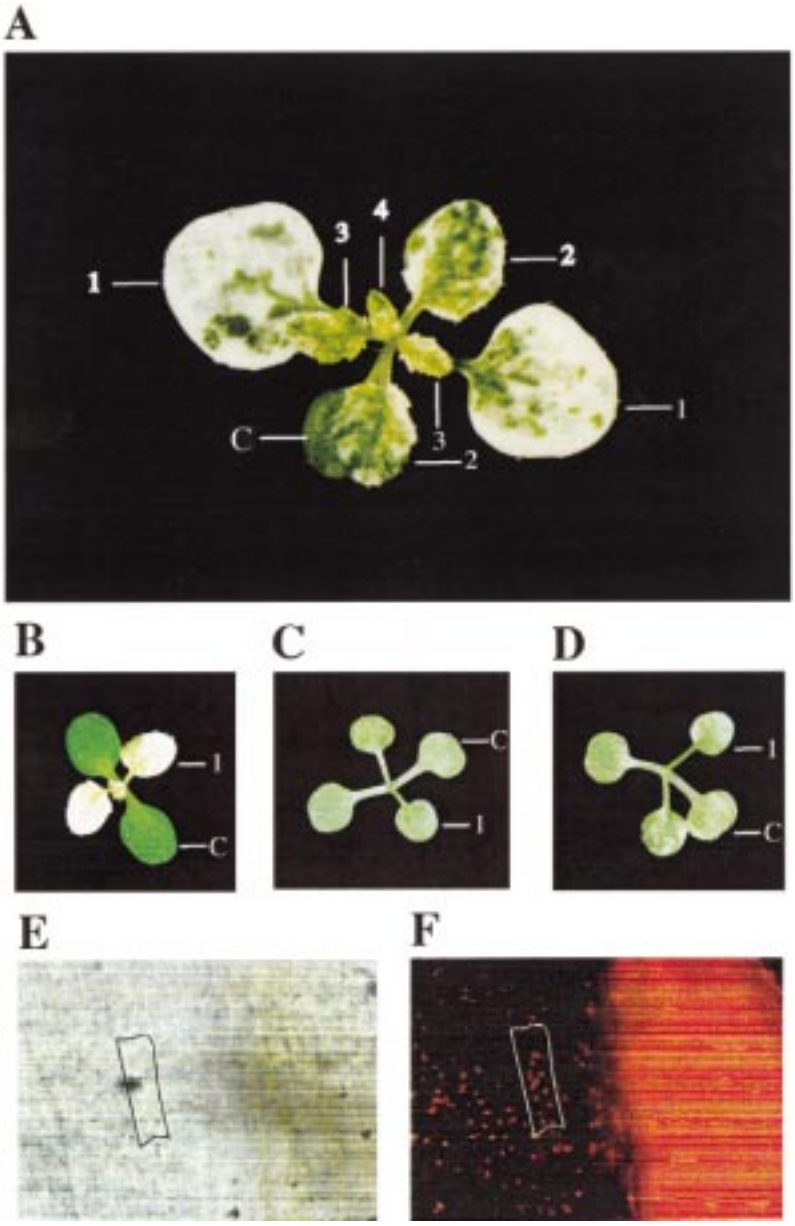

Figure 1. Phenotype of *var*2. (A) *var*2–1 plants with fully expanded first leaves 18 days after germination on soil (22°C, 100 μmol/m²/s; 16 h light/8 h dark). The order of emergence of the true leaves is shown (paired leaves "1" through "4"). C = cotyledons. **(B)** *var*2–1 with green cotyledons and young, yellow first leaves, 9 days after germination on soil (22°C, 100 μmol/m²/s; 16 h light/8 h dark). **(C)** Wild type (Col), 30 days after germination on soil (22°C, 15 μmol/m²/s; 16 h light/8 h dark). **(D)** *var*2–1, 30 days after germination on soil (22°C, 15 μmol/m²/s; 16 h light/8 h dark). **(E)** Phase micrograph of a portion of a "yellow" first leaf from *var*2–4 (*yellow variegated*). A typical cell is outlined. (Magnification 40×.) **(F)** The corresponding chlorophyll fluorescence image. Plastids with chlorophyll appear red. (Magnification 40×.)

Table 1. Alleles of *var*2

Allele	Mutant designation	Source[a]	Mutagen	Chlorophyll[b]
*var*2-1	VAR2	Martínez-Zapater	EMS	+
*var*2-2	VAR4	Martínez-Zapater	EMS	++
*var*2-3	69-2043b or CS3154	ABRC/Rédei	EMS	+++
*var*2-4	405-D-2357 (*yellow variegated*)	Rédei	X-ray	+++
*var*2-5	405-D-2569 ("greener" *immutans*)	Rédei	X-ray	++++

[a] The isolation of *var*2-1 and *var*2-2 is described in Martínez-Zapater (1993). The other alleles have not previously been reported. All alleles were isolated in the Columbia ecotype.

[b] ++++ → + (weakest allele → most severe allele): relative chlorophyll concentrations in first leaves that have recently attained full expansion.

Table 2. Reciprocal crosses between *var*2-4 and wild-type plants

	Observed phenotype			
	F_1		F_2[a]	
Parentals Female × Male	Green	Variegated	Green	Variegated
*var*2-4 × wild type	78	0	101	29
wild type × *var*2-4	61	0	312	88

[a] Chi-square analysis of F_2 data from both reciprocal crosses fits a 3:1 ratio ($P > .1$).

leaves. This phenomenon is likely a consequence of senescence-associated declines in photosynthetic capacity and chlorophyll content that occur during late dicot leaf development in fully expanded leaves (reviewed in Gepstein 1988).

*var*2–5 plants were used in the analyses in Figure 2 because this mutant is the least severe *var*2 allele (see below) and shows the most dramatic change in chlorophyll concentration as leaf development proceeds. In contrast, the most severe alleles (such as *var*2–1 in Figure 1A) have significantly less pigment than normal in fully expanded leaves.

*var*2 Allelic Series

The five alleles of *var*2 have visible differences in their degree of white-sector formation. To quantify these differences, we measured the chlorophyll concentrations of just fully expanded first leaves of the various alleles. The mutants were germinated and maintained under identical conditions in a growth chamber; the seedlings were exposed to continuous illumination. As illustrated in Figure 3, *var*2–1 leaves have significantly less chlorophyll than *var*2–5 first leaves, with the other alleles falling between these two extremes. Therefore we have classified *var*2–1 as the most severe allele and *var*2–5 as the least severe allele (Table 1).

Is *var*2 Blocked in Pigment Biosynthesis?

One possibility for the delayed accumulation of chlorophyll in *var*2 is that one of the steps of chlorophyll biosynthesis is partially blocked. To address this question we conducted δ-ALA feeding experiments. Feeding δ-ALA, the first committed precursor (step) in chlorophyll and heme biosynthesis allows the synthesis of porphyrins to proceed unregulated (Falbel and Staehelin 1994; Granick 1959; von Wettstein 1995). In wild-type plants in the dark, this treatment leads to an accumulation of protochlorophyllide, which can be photoconverted to chlorophyll if the plants are giv-

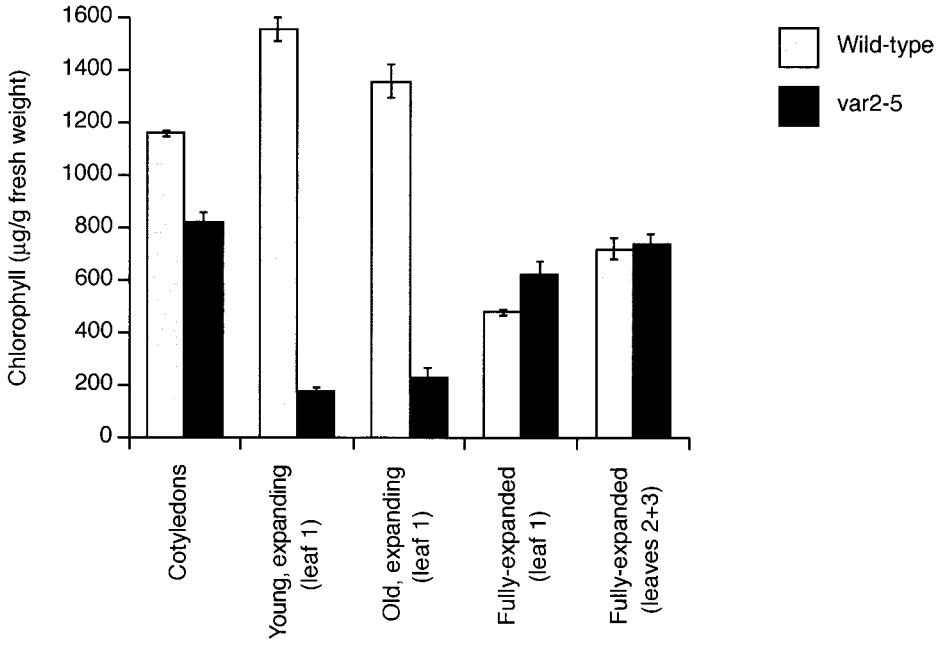

Figure 2. Chlorophyll concentrations during *var*2 development. Chlorophyll determinations were from the cotyledons and first leaves of *var*2–5 and wild-type plants. Three stages of first leaf ontogeny were examined: young expanding (yellow), old expanding, and fully expanded. Fully expanded second and third leaves were also examined. At least five different leaves of each type were sampled (means ± SE).

en light. In principle, plants with mutations in chlorophyll biosynthetic genes after δ-ALA formation but prior to protochlorophyllide reduction will accumulate precursors in this part of the pathway, thereby revealing the location of the block; such plants will not accumulate protochlorophyllide in the dark when exogenous δ-ALA is added.

In the case of *var*2, we reasoned that if a step in chlorophyll biosynthesis were inhibited, it might be easiest to detect in emerging yellow leaves of the most severe allele (*var*2–1). As shown in Figure 4A, protochlorophyllide accumulated to similar levels in detached mutant and wild-type leaves that were fed δ-ALA in the dark. This result indicates that the enzymes between δ-ALA and protochlorophyllide are present and functional at normal levels in the mutant. In contrast, when the leaves were fed δ-ALA in the light, much reduced levels of protochlorophyllide accumulated in the *var*2 and wild-type leaves (Figure 4B), indicating that protochlorophyllide oxidoreductase is functional in young *var*2 leaf tissues.

Although these sorts of experiments cannot pinpoint whether there are defects in the steps of chlorophyll biosynthesis between glutamate and δ-ALA, the data in Figure 4 suggest that the steps of chlorophyll biosynthesis downstream from the dehydratase are not impaired in the mutant. Consistent with this interpretation, HPLC analyses have revealed that pigment precursors do not accumulate in any *var*2 leaf tissues, including yellow first leaves and the white sectors of mature leaves (data not shown). These precursors include the colored chlorophyll and carotenoid intermediates, as well as the noncolored carotenoid precursor, phytoene, which is abundant in *im* white leaf tissues (Wetzel et al. 1994).

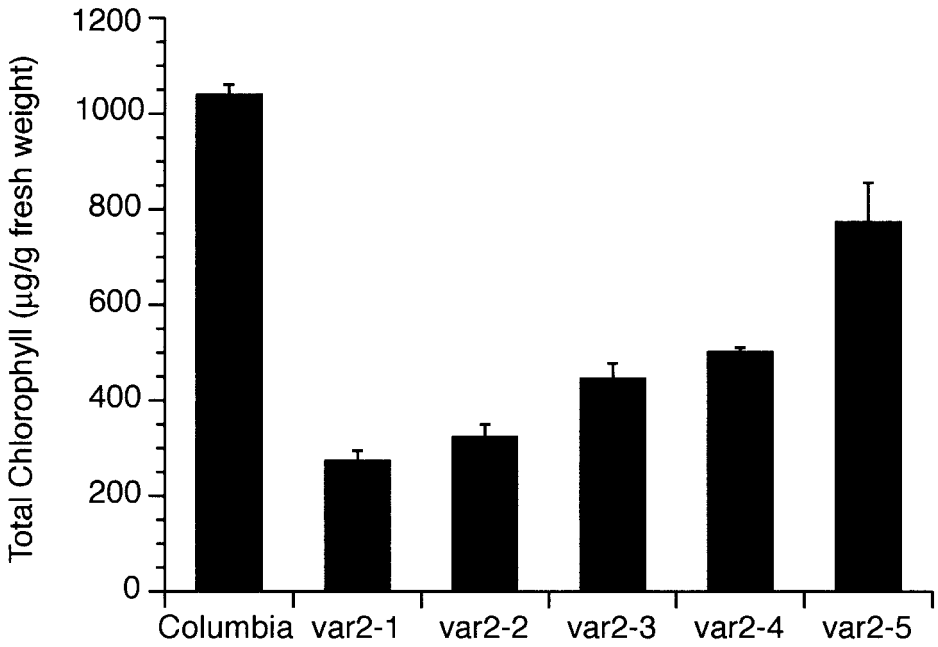

Figure 3. *var*2 allelic series. Chlorophyll concentrations were determined on just fully expanded leaves from wild-type and the five *var*2 alleles (means ± SE) growing on soil (22°C, 100 µmol/m²/s; 16 h light/8 h dark). At least five different leaves were examined per allele.

The Phenotype of *var*2 Is Plastid Autonomous

Figure 1E is a representative light micrograph of a section from an expanding, yellow leaf of *yellow variegated*; Figure 1F is the same section viewed via chlorophyll fluorescence microscopy. A typical cell is outlined. At this stage of mutant leaf development, nascent green sectors first become visible (the intense red chlorophyll autofluorescence) and cells in the yellow part of the leaf are sprinkled with autofluorescing bodies. During later expansion, the leaves become more green and the yellow sectors fade to white. Cells in the white sectors also contain low numbers of autofluorescing bodies, as in Figure 1F (data not shown).

Figure 5A shows normal chloroplasts, with their characteristic grana and starch grains, from an emerging wild-type leaf of *yellow variegated.* Morphologically normal chloroplasts are also found in the green sectors of the mutant (data not shown). Most plastids in emerging yellow leaves lack internal membrane structures (Figure 5B). These plastids are significantly larger than proplastids (3 µm versus 0.5–1 µm; Bowman 1994), are highly vacuolated, and contain numerous bodies that resemble plastoglobuli, which are lipid droplets that contain quinones, tocopherols, and carotenoids (Gunning and Steer 1996; Lichtenthaler 1968). The emerging yellow leaves

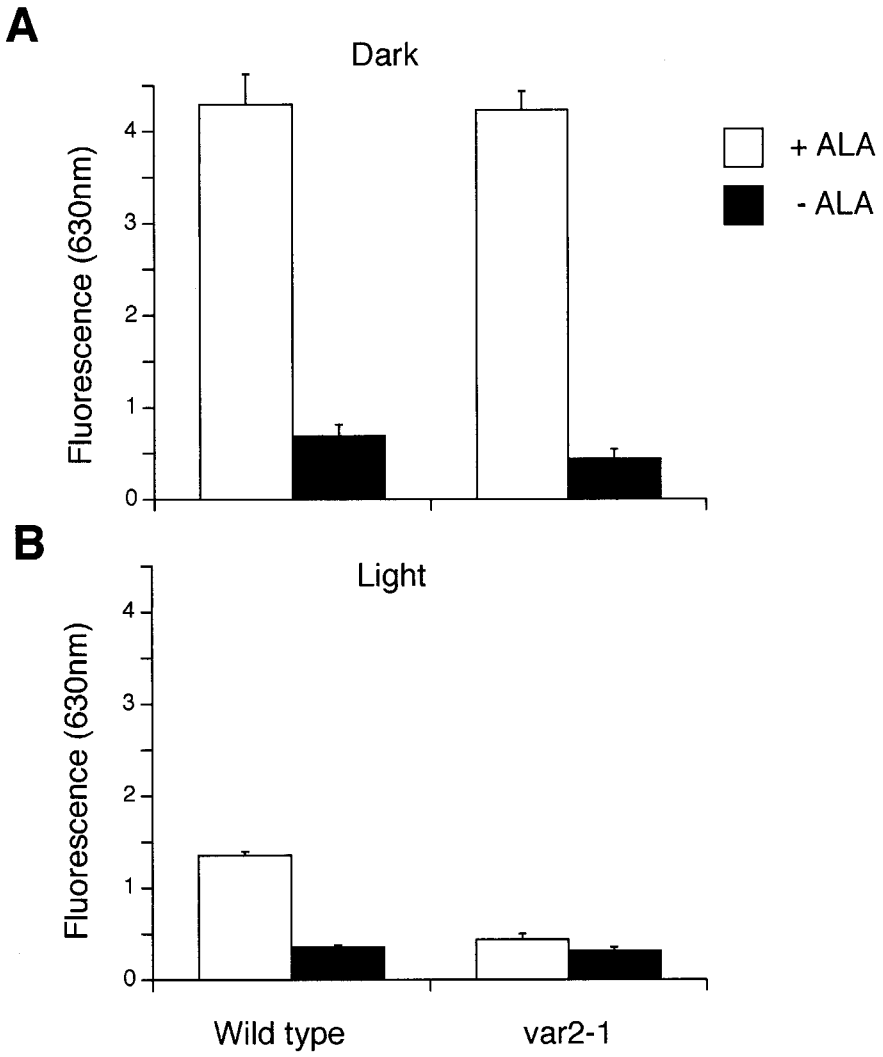

Figure 4. δ-ALA feeding experiment. Detached, first leaves (0.1 g) from young *var*2–1 and wild-type plants were fed with 10 mM δ-ALA (pH 7.0) in **(A)** darkness or **(B)** light for 18 h. The pigments were extracted and protochlorophyllide concentrations were determined by measuring fluorescence at 630 nm (excitation 440 nm). Each bar represents the means (±SE) of at least five separate experiments.

also contain plastids that appear to be in varying stages of development, ranging from those that are highly vacuolated with rudimentary lamellar structures (Figure 5C, plastid on the left) to those that resemble chloroplasts (Figure 5D, top right). Some cells in the yellow leaves are heteroplastidic and contain plastids with different morphologies (Figures 5C and 5D). This observation confirms the suggestion from the fluorescence analyses (Figure 1F) that pigmented plastids represent a small fraction of the plastid pool in the yellow sectors. Although heteroplastidic cells are also found in the white sectors of mature leaves, most cells in these sectors are homoplastidic and contain plastids with a morphology similar to those in Figure 5B, that is, highly vacuolated and lacking internal membrane structures (data not shown).

Considered together with the pigment analyses, the fluorescence and ultrastructural analyses suggest that the yellow color of emerging *var*2 leaves may be, in part, an optical illusion caused by green speckles (the red autofluorescing bodies) against a white background. Yet some of the color may also be contributed by low concentrations of chlorophyll present in plastids with poorly developed internal membrane structures.

Light and Temperature Sensitivity of *var*2

To examine whether white-sector formation in *var*2 is sensitive to light intensity, we grew mutant and wild type plants on soil at 15 versus 100 μmol/m²/s; the temperature was maintained at 22°C. Figure 1A,B shows the growth of *var*2–1 (the most severe allele) at the higher light intensity 9 and 18 days after germination. Wild-type plants, even though they were green, had a similar number of leaves and a similar morphology under these conditions at each of these time points (data not shown). In contrast, Figure 1C,D shows wild-type and *var*2 plants, respectively, at the lower light intensity 30 days after germination. While the two plants are morphologically similar to one another at this light intensity, the *var*2 plants display little variegation at the lower light. We conclude that light intensity is able to influence plant growth rates and the expressivity of variegation in *var*2.

To examine the sensitivity of *var*2 to temperature, we grew *var*2 at 28°C versus 22°C; illumination was maintained at 100 μmol/m²/s. We observed that at both temperatures the plants grew at very similar rates, as monitored by leaf emergence, leaf number, and days to flowering (data not shown). However, both sets of plants grew more slowly at the higher temperature. Whereas at the lower temperature a significant amount of variegation was observed in the *var*2 plants, at the higher temperature, emerging yellow first leaves of *var*2 had more green sectors and fully expanded (mature) leaves were nearly all green. Some mature leaves were completely green and indistinguishable from wild-type leaves at this temperature.

We conclude that light and temperature affect the growth rates of wild-type and *var*2 *A. thaliana,* and that these factors can also modulate the extent of variegation that occurs in *var*2. This conclusion suggests that variegation can be influenced by the rate of plant growth, with green sector formation being promoted by those factors that depress growth rates. Our results are consistent with those of Martínez-Zapater (1993), who reported that light and temperature can influence the extent of sector formation in *var*2. However, he did not examine wild type plants, nor did he monitor plant growth.

Discussion

The *var*2 Mutant Is "Plastid Autonomous"

Plastids in *var*2 leaves have different morphologies. Although cells in the white and yellow sectors can be homoplastidic for plastids lacking internal membrane structures, cells in these sectors are frequently heteroplastidic. Heteroplastidic cells usually contain plastids that appear to be in varying stages of thylakoid membrane biogenesis, ranging from those that are highly

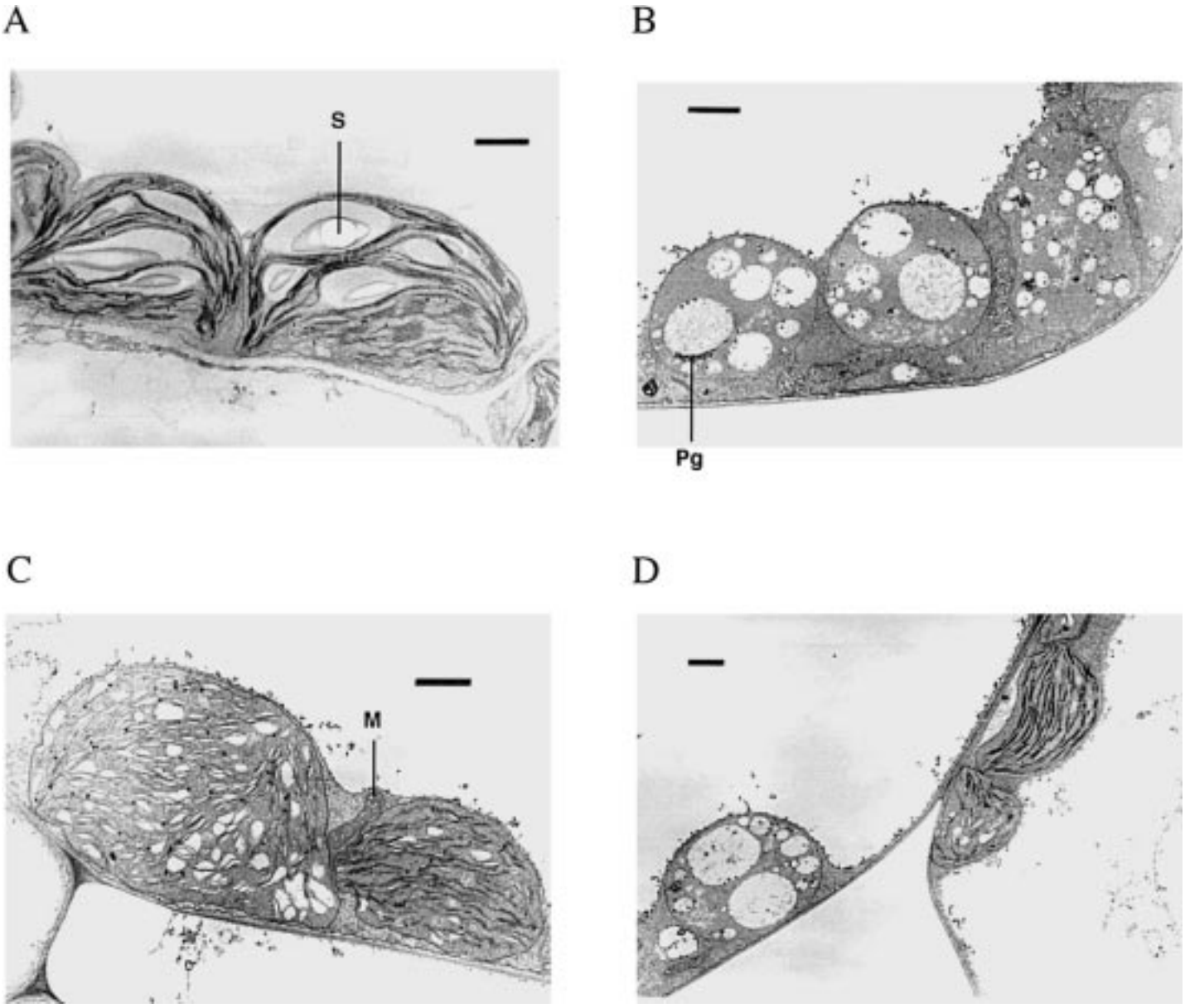

Figure 5. Electron micrographs of plastids from *var*2 leaves. **(A)** A representative chloroplast from wild type first leaves. **(B–D)** Representative plastids from emerging, yellow first leaves of *var*2–4 (*yellow variegated*). Bar = 1 μm. Pg = plastoglobuli; S = starch; M = mitochondrion.

vacuolated and lacking lamellae, to those that resemble chloroplasts and that probably correspond to the red autofluorescing bodies in Figure 1F. The finding of heteroplastidic cells indicates that *var*2 affects the plastids in a cell unequally and that its expression is "plastid autonomous."

Heteroplastidic "mixed" cells are rare in plants and are thought to be intermediates in a process of sorting out to form clones (sectors) of cells containing either all-white or all-green plastids (Gillham et al. 1991; Tilney-Bassett 1984). The white plastids in such cells are permanently defective and thus the plastid defect is maternally inherited. Permanently defective plastids in variegated plants can arise either by mutations in the plastid DNA, as in the well-known plastome mutants of *Oenothera* (e.g., Stubbe and Herrmann 1982), or by mutations in the nuclear DNA that permanently affect plastid function. Examples of the latter include the *iojap* mutant of maize (Coe et al. 1988; Han et al. 1992; Rhoades 1943; Walbot and Coe 1979) and the *albostrians* mutant of barley (e.g., Hess et al. 1992, 1994). There are also cases where permanently defective mitochondria are produced by nuclear recessive gene products that cause rearrangements in the mitochondrial genome. The defective mitochondria are maternally inherited and secondarily affect plastid function and morphology. Examples of this type of variegation include *chloroplast mutator* in *Arabidopsis* (Martínez-Zapater et al. 1992; Rédei 1973; Sakamoto et al. 1996) and *NCS* (nonchromosomal stripe) in maize (e.g., Gu et al. 1993; Newton and Coe 1986; Roussell et al. 1991).

In contrast to other nuclear gene-induced variegations, defective plastids are not maternally inherited in *var*2. Nonmaternal inheritance suggests that *var*2 expression is plastid autonomous due to an "extrinsic" nuclear-cytoplasmic factor (such as the *var*2 gene product itself) rather than to an "intrinsic" organelle factor that causes a permanent defect (such as an organelle DNA mutation caused by

*var*2 action). The possibility that plastids can respond differently to cytoplasmic factors was first suggested in 1943 by Rhoades in his classic studies on *iojap* (Granick 1955; Rhoades 1943). *var*2 is thus an ideal system to explore the underlying mechanism(s) of this phenomenon.

Similar to *var*2, *im* is plastid autonomous, and defective *im* plastids are not maternally transmitted (Wetzel et al. 1994). *var*2, along with *im,* thus defines a novel class of nuclear gene-induced variegation mutant. The lack of maternal inheritance in these mutants may suggest that defective plastids are capable of being restored to normal during or following reproduction. Alternatively, such plastids may be excluded from reproductive cells.

Mechanism of *var*2 Variegation

The data in this report are consistent with the hypothesis that the *VAR*2 gene product is a plastid-localized protein required for normal thylakoid membrane assembly and chloroplast differentiation. We would consequently predict that nonpigmented plastids have less of the protein than chloroplasts. Because *var*2 cotyledons appear normal, it is also likely that the *VAR*2 gene product first becomes active during the chloroplast differentiation process in true leaves. Based on our interpretation of the different plastid types in the yellow and white sectors of the mutant as intermediates in chloroplast biogenesis, we consider it probable that plastid development is delayed in *var*2. However, we cannot rule out the possibility that some of the plastids in *var*2 leaves are photooxidized, or partially so (Meehan et al. 1996; Susek et al. 1993).

Martínez-Zapater (1993) reported that the degree of white-sector formation in *var*2 could be qualitatively modulated by light and temperature. We have found that both of these factors also appear to affect plant growth rates. In fact, at either high temperatures or low light conditions, growth rates appear to be retarded and *var*2 leaves are nearly all green. Therefore it is possible that the random, nonclonal variegation in *var*2 plants could arise as a consequence of an interplay between factors that determine the temporal and spatial aspects of *VAR*2 gene expression and factors that specify rates of cell and plastid division (increased by high light and low temperature). For example, if *VAR*2 expression was limited to cells in the expanding leaf, enhanced rates of cell and plastid division during the expansion phase could result (in the mutant background) in clones (sectors) of cells, most of whose plastids lack the necessary amount of this protein for normal chloroplast biogenesis. This model/hypothesis assumes that the *var*2 alleles we have examined are not null, and that they produce partially active *var*2 (mutant) proteins.

Regardless of the precise mechanism of *var*2 variegation, an interplay between gene expression and cell division is known to contribute to the specification of developmental patterns in animal species, such as *Drosophila.* A precedent for our working model is also provided by studies on anthocyanin variegation in maize vegetative organs (Cocciolone and Cone 1993; Cone et al. 1993a,b) and mustard cotyledons (Nick et al. 1993). These studies have suggested that there is a stochastic element involved in the light regulation of anthocyanin accumulation, resulting in a patchiness of expression. It has been suggested that this may be due to epigenetic factors (e.g., methylation) and/or to cell-to-cell variations in light perception. Although we cannot rule out similar epigenetic explanations for *var*2 variegation, elements of our working model can be tested once *var*2 is cloned and sequenced. We are currently using map-based methods to isolate the gene.

References

Bowman J, 1994. Arabidopsis: An atlas of morphology and development. New York: Springer-Verlag.

Cocciolone SM and Cone KC, 1993. Pl-Bh, an anthocyanin regulatory gene of maize that leads to variegated pigmentation. Genetics 135:575–588.

Coe EH Jr, Thompson D, and Walbot V, 1988. Phenotypes mediated by the *iojap* genotype in maize. Am J Bot 75:634–644.

Cone KC, Cocciolone SM, Burr FA, and Burr B, 1993a. Maize anthocyanin regulatory gene pl is a duplicate of c1 that functions in the plant. Plant Cell 5:1795–1805.

Cone KC, Cocciolone SM, Moehlenkamp CA, Weber T, Drummond BJ, Tagliani LA, Bowen BA, and Perrot GH, 1993b. Role of the regulatory gene pl in the photocontrol of maize anthocyanin pigmentation. Plant Cell 5: 1807–1816.

Falbel TG and Staehelin LA, 1994. Characterization of a family of chlorophyll-deficient wheat (*Triticum*) and barley (*Hordeum vulgare*) mutants with defects in the magnesium-insertion step of chlorophyll biosynthesis. Plant Physiol 104:639–648.

Gepstein S, 1988. Photosynthesis. In: Senescence and aging in plants (Noodén LD and Leopold AC, eds). San Diego: Academic Press; 85–109.

Gillham NW, Boynton JE, and Harris EH, 1991. Transmission of plastid genes. Cell Cult Somatic Cell Genet Plants 7A:55–92.

Giuliano G, Bartley GE, and Scolnik PA, 1993. Regulation of carotenoid biosynthesis during tomato development. Plant Cell 5:379–387.

Granick S, 1955. Die plastiden und chondriosomen. In: Encyclopedia of plant physiology, vol. I (Ruhland W, ed). Berlin: Springer Verlag; 507–564.

Granick S, 1959. Magnesium porphyrin formed by barley seedlings treated with δ-aminolevulinic acid. Plant Physiol 34:S-xviii.

Gu J, Miles D, and Newton K, 1993. Analysis of leaf sectors in the NCS6 mitochondrial mutant of maize. Plant Cell 5:963–971.

Gunning BES and Steer MW, 1996. Plant cell biology, structure and function. Boston: Jones and Bartlett.

Han C-D, Coe EH, and Martienssen RA, 1992. Molecular cloning and characterization of *iojap* (*ij*), a pattern striping gene of maize. EMBO J 11:4037–4046.

Hess WR, Müller A, Nagy F, and Börner T, 1994. Ribosome-deficient plastids affect transcription of light-induced nuclear genes: genetic evidence for a plastid-derived signal. Mol Gen Genet 242:305–312.

Hess WR, Schendel R, Rüdiger W, Fieder B, and Börner T, 1992. Components of chlorophyll biosynthesis in a barley albina mutant unable to synthesize δ-aminolevulinic acid by utilizing the transfer RNA for glutamic acid. Planta 188:19–27.

Hiscox JD and Israelstam GF, 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. Can J Bot 57:1332–1334.

Hodgins RR and van Huystee RB, 1986. Rapid simultaneous estimation of protoporphyrin and Mg-porphyrins in higher plants. J Plant Physiol 125:311–323.

Horner HT and Wagner BL, 1980. The association of druse crystals with the developing stomium of *Capsicum annum* (Solanaceae) anthers. Am J Bot 67:1347–1360.

Lichtenthaler HK, 1968. Plastoglobulae and the fine structure of plastids. Endeavor 27:144–149.

Martínez-Zapater JM, 1993. Genetic analysis of variegated mutants in *Arabidopsis.* J Hered 84:138–140.

Martínez-Zapater JM, Gil P, Capel J, and Somerville CR, 1992. Mutations at the *Arabidopsis CHM* locus promote rearrangements of the mitochondrial genome. Plant Cell 4:889–899.

Meehan L, Harkins K, Chory J, and Rodermel S, 1996. *Lhcb* transcription is coordinated with cell size and chlorophyll accumulation. Studies on fluorescence-activated, cell-sorter-purified single cells from wild type and *immutans Arabidopsis thaliana.* Plant Physiol 112: 953–963.

Mullet J, 1988. Chloroplast development and gene expression. Annu Rev Plant Physiol Plant Mol Biol 39:475–502.

Newton KJ and Coe EH Jr, 1986. Mitochondrial DNA changes in abnormal growth mutants of maize. Proc Natl Acad Sci USA 83:7363–7366.

Nick P, Ehmann B, Furuya M, and Schäfer E, 1993. Cell communication, stochastic cell responses, and anthocyanin pattern in mustard cotyledons. Plant Cell 5:541–552.

Rédei GP, 1963. Somatic instability caused by a cysteine-sensitive gene in *Arabidopsis.* Science 139:767–769.

Rédei GP, 1967. Biochemical aspects of a genetically determined variegation in *Arabidopsis.* Genetics 56: 431–443.

Rédei GP, 1973. Extra-chromosomal mutability determined by a nuclear gene locus in *Arabidopsis.* Mutat Res 18:149–162.

Rédei GP, 1975. *Arabidopsis* as a genetic tool. Annu Rev Genet 9:111–127.

Rhoades MM, 1943. Genic induction of an inherited cytoplasmic difference. Proc Natl Acad Sci USA 29:327–329.

Roussell DL, Thompson DL, Pallardy SG, Miles D, and Newton KJ, 1991. Chloroplast structure and function is altered in the NCS2 maize mitochondrial mutant. Plant Physiol 96:232–238.

Runge S, van Cleve B, Lebedev N, Armstrong G, and

Apel K, 1995. Isolation and classification of chlorophyll-deficient xantha mutants *of Arabidopsis thaliana.* Planta 197:490–500.

Sakamoto W, Kondo H, Murata M, and Motoyoshi F, 1996. Altered mitochondrial gene expression in a maternal distorted leaf mutant of *Arabidopsis* induced by *chloroplast mutator.* Plant Cell 8:1377–1390.

Stubbe W and Herrmann RG, 1982. Selection and maintenance of plastome mutants and interspecific genome/plastome hybrids from *Oenothera.* In: Methods in chloroplast molecular biology (Edelman M, Hallick RB, and Chua N-H, eds). New York: Elsevier Medical; 149–165.

Susek RE, Ausubel FM, and Chory J, 1993. Signal transduction mutants of *Arabidopsis* uncouple nuclear *CAB* and *RBCS* gene expression from chloroplast development. Cell 74:787–799.

Susek RE and Chory J, 1992. A tale of two genomes: role of a chloroplast signal in coordinating nuclear and plastid genome expression. Aust J Plant Physiol 19: 387–399.

Taylor WC, 1989. Regulatory interactions between nuclear and plastid genomes. Annu Rev Plant Physiol Plant Mol Biol 40:211–233.

Tilney-Bassett RAE, 1984. The genetic evidence for nuclear control of chloroplast biogenesis in higher plants. In: Chloroplast biogenesis (Ellis RJ, ed). Cambridge: Cambridge University Press; 13–50.

von Wettstein D, Gough S, and Kannangara CG, 1995. Chlorophyll biosynthesis. Plant Cell 7:1039–1057.

Walbot V and Coe EH Jr, 1979. Nuclear gene *iojap* conditions a programmed change to ribosome-less plastids in *Zea mays.* Proc Natl Acad Sci USA 76:2760–2764.

Wetzel CM, Jiang C-Z, Meehan LJ, Voytas DF, and Rodermel SR, 1994. Nuclear-organelle interactions: the *immutans* variegation mutant of *Arabidopsis* is plastid autonomous and impaired in carotenoid biosynthesis. Plant J 6:161–175.

Wetzel CM and Rodermel SR, 1998. Regulation of phytoene desaturase expression is independent of leaf pigment content in *A. thaliana.* Plant Mol Biol 37:1045–1053.

Received February 17, 1998
Accepted August 31, 1998

Corresponding Editor: Norman Weeden