Plant, Cell and Environment (2007) **30**, 350–365 doi: 10.1111/j.1365-3040.2006.01630.x

Variegation mutants and mechanisms of chloroplast biogenesis

FEI YU, AIGEN FU*, MANEESHA ALURU, SUNGSOON PARK*, YANG XU, HUIYING LIU, XIAYAN LIU, ANDREW FOUDREE, MILLY NAMBOGGA & STEVEN RODERMEL

Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011

ABSTRACT

Variegated plants typically have green- and white-sectored leaves. Cells in the green sectors contain normal-appearing chloroplasts, whereas cells in the white sectors lack pigments and appear to be blocked at various stages of chloroplast biogenesis. Variegations can be caused by mutations in nuclear, chloroplast or mitochondrial genes. In some plants, the green and white sectors have different genotypes, but in others they have the same (mutant) genotype. One advantage of variegations is that they provide a means of studying genes for proteins that are important for chloroplast development, but for which mutant analysis is difficult, either because mutations in a gene of interest are lethal or because they do not show a readily distinguishable phenotype. This paper focuses on *Arabidopsis* variegations, for which the most information is available at the molecular level. Perhaps the most interesting of these are variegations caused by defective nuclear gene products in which the cells of the mutant have a uniform genotype. Two questions are of paramount interest: (1) What is the gene product and how does it function in chloroplast biogenesis? (2) What is the mechanism of variegation and why do green sectors arise in plants with a uniform (mutant) genotype? Two paradigms of variegation mechanism are described: *immutans* (*im*) and *variegated2* (*var2*). Both mechanisms emphasize compensating activities and the notion of plastid autonomy, but redundant gene products are proposed to play a role in *var2*, but not in *im*. It is hypothesized that threshold levels of certain activities are necessary for normal chloroplast development.

Key-words: *Arabidopsis*; chloroplast mutator; *immutans*; maternal inheritance; nuclear-organelle interactions; retrograde signals; *var*2.

INTRODUCTION

Variegation mutants have been defined as 'any plant that develops patches of different colors in its vegetative parts' (Kirk & Tilney-Bassett 1978). As early as 1868, Darwin classified 'sports' (spontaneous mutations) according to the plant organ in which they appeared to have their primary effect; many sports were variegations (Tilney-Bassett 1986). Some of the most common variegations have green and white (or yellow) sectors in normally green tissues and organs of the plant. Cells in the green sectors typically contain normal-appearing chloroplasts; cells in the white (or yellow) sectors contain plastids that are deficient in chlorophyll and/or carotenoid pigments. These plastids appear to be photooxidized or blocked at various steps of chloroplast biogenesis because they frequently lack organized internal membrane structures and/or contain only rudimentary lamellae. Despite their widespread occurrence in nature, relatively few variegations have been characterized at the molecular level.

Variegation mutants have played a prominent role in the history of genetics (Tilney-Bassett 1975; Kirk & Tilney-Bassett 1978). As a notable example, Correns and Baur observed in the early 1900s that transmission of the variegation trait does not always obey Mendel's laws, paving the way for the discovery of non-Mendelian (maternal) inheritance (reviewed by Granick 1955). Whereas variegations have long been associated with differences in plastid form and function, it was not possible to gain insight into the molecular basis of this phenomenon until the 1960s and early 1970s, when compelling molecular evidence was presented that mitochondria and chloroplasts contain their own DNA and protein synthesis systems, and that organellar proteins are the products of genes in the chloroplast and mitochondrion, as well as the nucleus (reviewed by Bogorad 1981). Today, it is well established that nuclear-encoded organellar proteins are translated as precursors on 80S ribosomes in the cytosol and transported into the organelle posttranslationally, whereas organelle-encoded proteins are translated on prokaryotic-like 70S ribosomes in the organelle itself (reviewed by Goldschmidt-Clermont 1998). Gene expression in the organelle and nucleus–cytoplasm is coordinated and integrated by a variety of poorly understood anterograde (nucleus-to-organelle) and retrograde (organelle-to-nucleus) signalling mechanisms (reviewed by León, Arroyo & Mackenzie 1998; Rodermel 2001; Pfannschmidt 2003; Nott *et al*. 2006).

Correspondence: S. Rodermel. Fax: 515 294-1337; e-mail: rodermel@iastate.edu

*Current Address: Department of Plant & Microbial Biology, University of California Berkeley, Berkeley, CA 94720

MECHANISMS OF VARIEGATION

Variegations can arise by many different mechanisms (reviewed by Tilney-Bassett 1975; Kirk & Tilney-Bassett 1978). Some variegations are induced by external agents and are not heritable. For instance, chlorotic leaf sectors can be generated by preferential shading, pathogen attack and nutritional deficiencies. Heritable variegations, on the other hand, arise from mutations in nuclear, plastid and/or mitochondrial genes that result in a failure of plastids to accumulate photosynthetic pigments, either directly or indirectly, producing sectors with cells containing white or yellow plastids. The abnormal plastids are often, but not always, 'permanently defective' and inherited in a non-Mendelian fashion, i.e. maternally (in the majority of angiosperms) or biparentally (Tilney-Bassett 1975; Connett 1987). In cases of maternal inheritance, the probability of transmission of a permanently defective organelle is related to the extent of variegation of the mother plant. In many cases, the defective plastids (and the cells that contain them) replicate normally, or nearly so, and sort out to produce clones of cells containing morphologically normal chloroplasts (green sectors) or abnormal plastids (white or yellow sectors) (reviewed by Kirk & Tilney-Bassett 1978; Hagemann 1986; Tilney-Bassett 1986, 1989). Preferential replication of green versus white plastids (or cells) has also been documented (Hagemann 1986; Stubbe 1989; Park *et al*. 2000).

There are two major types of variegation depending on the genotypes of the white and green sectors. These types of variegations can be caused by mutations in nuclear, plastid or mitochondrial genes. In the first, cells in the green sectors have a wild-type (WT) genotype, while cells in the white sectors have a mutant genotype. Prominent examples include the following:

1 **Chimerism** occurs when different histological regions of a plant meristem, and consequently the tissues that derive from them, have different genotypes (reviewed by Kirk & Tilney-Bassett 1978; Tilney-Bassett 1986).
2 **Transposable element activity** generates variegated plants when insertion of a transposon interrupts a nuclear gene required for normal chloroplast biogenesis (white sectors), while element excision (or silencing) reconstitutes WT gene expression (green sectors). These are often termed *mutable alleles*. Mutable alleles were first described in maize by McClintock (reviewed by Federoff 1989; Feschotte, Jiang & Wessler 2002) and have been used to clone a variety of genes in maize and other species. Some well-known examples of mutable alleles of genes important for chloroplast biogenesis include maize *bundle sheath defective 1* (*bsd1*), which is allelic to *golden2* (Langdale & Kidner 1994; Hall *et al*. 1998), *bsd2* (Brutnell *et al*. 1999), *hcf106* (Martienssen *et al*. 1989; Settles *et al*. 2001) and *leaf permease1* (*lpe1*) (Schultes *et al*. 1996); *defective chloroplasts and leaves* (*dcl*) in tomato (Keddie *et al*. 1996; Bellaoui, Keddie & Gruissem 2003); *olive* and *differentiation and greening* (*dag*) in *Antirrhinum* (Hudson *et al*. 1993; Chatterjee *et al*. 1996; Chatterjee & Martin 1997); and *pale-yellow-leaf variegated* (*pyl-v*) in rice (Tsugane *et al*. 2006). In *Arabidopsis*, mutable alleles have been reported in transposon-tagging experiments using heterologous elements, such as the autonomous maize En-1 element, or the maize Dissociation (Ds) transposable element/Activator (Ac) transposase system (e.g. Klimyuk *et al*. 1995; Wisman *et al*. 1998).
3 **RNA silencing** encompasses a wide range of phenomena in which gene expression is regulated by small RNAs (ca. 21–26 nt) produced from dsRNAs or stem-loop RNA precursors (reviewed by Meins, Si-Ammour & Blevins 2005). These RNAs guide the cleavage of target gene RNAs (as with RNAi, antisense, or cosuppression), block their translation or induce the methylation of target genes. All of these mechanisms can produce variegation when a nuclear gene required for chloroplast biogenesis is silenced in some cells but not in others. An early, dramatic example of RNA silencing was the production of variegated flowers in transgenic petunia that contain an antisense *chalcone synthase* gene (e.g. van der Krol *et al*. 1988, 1990). *IspH* transgene-induced gene silencing is a recent example of leaf variegation in *Arabidopsis* (Hsieh & Goodman 2005); IspH catalyses the last step of the plastid-localized non-mevalonate pathway of isoprenoid biosynthesis.
4 **Plastome mutators** are nuclear genes that cause mutations in chloroplast DNA (reviewed by Tilney-Bassett 1975; Börner & Sears 1986). For example, the *plastome mutator* (*pm*) gene of *Oenothera* (evening primrose) causes insertions and deletions in plastid genes that result in template slippage and the generation of permanently defective, biparentally inherited plastids (Chang *et al*. 1996; Stoike & Sears 1998; GuhaMajumdar, Baldwin & Sears 2004). 'Mixed cells' that contain plastids with mutant and normal plastid genomes (plastomes) sort out to form homoplasmic clones of plastids and cells containing all-mutant (white) or all-WT (green) plastid DNAs.
5 **Plastome mutations** can arise spontaneously or following treatment with various chemical mutagens (reviewed by Tilney-Bassett 1975; Börner & Sears 1986; GuhaMajumdar *et al*. 2004). Plastome mutants can also be produced by chloroplast transformation (e.g. Maliga 2004). Such 'transplastomic' lines can be engineered by the integration of an antibiotic cassette into the plastome by homologous recombination via flanking plastid DNA sequences in the vector; the transformed plastid DNAs subsequently sort out to form homoplasmic lines. It might be anticipated that mutations in many plastid genes, regardless of how they are generated, would give rise to defective plastids because plastomes primarily code for core components of the photosynthetic apparatus and for proteins involved in plastid gene expression (reviewed by Goldschmidt-Clermont 1998). As with plastome mutators, variegations arise when these genomes sort out to form homoplasmic clones of plastids and cells.
6 Some **mitochondrial genome mutations** cause variegation as a secondary consequence of defective mitochondria, i.e. the mitochondrial defect leads to lesions in

chloroplast biogenesis or maintenance, generating white or yellow plastids. This is presumably caused by a disruption in the normal flow of metabolites and regulatory information from mitochondria to chloroplasts (and vice versa) (Raghavendra, Padmasree & Saradadevi 1994). The *non-chromosomal stripe* (*NCS*) mutants of maize are well-known examples of this type of variegation (Newton & Coe 1986). These mutants have abnormal growth phenotypes and are heteroplasmic for mutant and normal mtDNAs. During development, defective mitochondria sort out to form stripes containing cells that are homoplasmic (or nearly so) for WT (green stripes) or mutant DNAs (pale green or yellow stripes); the defective mitochondria are maternally inherited (Newton & Coe 1986; Newton *et al*. 1990). Five *NCS* mutants have been characterized at the molecular level and all are caused by small deletions in essential mitochondrial genes (reviewed by Gabay-Laughnan & Newton 2005). All of the *NCS* mutations arose in the Wf9 (or Wf9-related) nuclear background, and while it is thought that their generation is caused by nuclear gene mutations, the causative alleles have not been identified (Newton, personal communication). *NCS*-like mutations are found in other higher plant species, including tobacco (Bonnett *et al*. 1993; Gutierres *et al*. 1997), tomato (Bonnema *et al*. 1995) and *Arabidopsis* (Sakamoto *et al*. 1996).

7 **Plastid–nucleus incompatibility** can cause variegation. A well-known example of this phenomenon is *Oenothera* (evening primrose) (reviewed by Hagemann 1986; Stubbe 1989). Each species of *Oenothera* is distinguished by a certain 'plastid type' and 'nuclear type', and 'incompatibility' between them occurs when they are combined in novel combinations in interspecific crosses. Incompatibility manifests itself in developmental disturbances that are frequently accompanied by pigment deficiencies (including variegations) and, in extreme cases, by an inhibition of plastid and cell reproduction (e.g. Glick & Sears 1994).

In the second major type of variegation, the cells of the mutant have a uniform mutant genotype, but the mutant phenotype is expressed in only a subset of cells (white sectors). Variegations of this sort are typically induced by nuclear recessive genes. Examples of this type of variegation have been known since ca. 1921 (reviewed by Hagemann 1986). One of the earliest (and a textbook case of maternal inheritance) is *iojap*, a maize striping mutant (Walbot & Coe 1979; Coe, Thompson & Walbot 1988). *iojap* plants have a homozygous recessive genotype, but the phenotype is expressed only in the white stripes; the green stripes appear normal. The plastids in the white sectors of *iojap* lack 70S ribosomes and are permanently defective because plastid ribosome biogenesis requires the ability to translate 70S ribosomal proteins encoded in the plastome. Hence, once a plastid has lost its ribosomes, it cannot regain them even if returned to a WT nuclear background. *iojap* has been cloned and appears to code for a component of the 50S subunit of the plastid ribosome (Han, Coe & Martienssen 1992; Han & Martienssen 1995); the reason for the striping is not understood. The well-known *Saskatoon* and *albostrians* mutants of barley are other examples of striping mutants that lack plastid 70S ribosomes because of nuclear recessive genes (Börner & Sears 1986; Hagemann 1986; Hess *et al*. 1994; Yaronskaya *et al*. 2003); neither of these genes has been cloned.

Although a number of nuclear gene-induced variegations have been reported, the primary lesions and mechanism of variegation are understood in very few of them. The best understood examples come from *Arabidopsis*. Hence, the remainder of this paper will focus on *Arabidopsis* variegations and in particular on those induced by nuclear gene products. Several questions are of particular relevance in surveying each mutant. Firstly, it is of interest to know the identity of the gene product defined by each mutant locus, how it functions in plastid biogenesis and why plastids are defective in the mutant. Secondly, it is important to know the mechanism of variegation. Why are the mutants variegated rather than albino or uniformly pigmented? The fact that so few variegations of this type have been documented raises the possibility that they have an underlying variegation mechanism(s) in common.

In addressing these questions, two *Arabidopsis* variegations will be discussed in greater detail than the others – *immutans* (*im*) and *variegated2* (*var2*). These mutants have been characterized more fully than others and should provide a foundation about the basic methodologies used to study variegation mutants. In addition, these mutants represent two different paradigms of variegation mechanism. The discussion will also be limited to variegations that are present throughout the life of the plant, and will exclude a large number of slow-greening or virescent mutants that are sectored only during an early part of their development (e.g. López-Juez *et al*. 1998).

ARABIDOPSIS VARIEGATIONS

Mutagenesis experiments in *Arabidopsis thaliana* typically generate a low frequency of colour mutants. For instance, Reiter *et al*. (1994) identified nearly 300 colour mutants in a collection of ~ 8000 T-DNA-tagged plants (3.75% frequency). These mutants had a uniform pigmentation and no variegations were reported. Of 78 colour mutants recovered in a collection of 10 950 T-DNA- and En-tagged lines (0.75% frequency), four were variegations (0.04% frequency) (Wisman *et al*. 1998; Maiwald, unpublished observations). Recent large-scale phenotyping of 4000 transposon insertion lines, each with a Ds element in a gene coding region, resulted in 139 mutants with reproducible, visual phenotypes in their aerial organs (Kuromori *et al*. 2006). Five of these were variegations and four were reticulate (~ 0.2% overall frequency).

In contrast to the relatively low percentage of variegations that arise in tagging experiments, McKelvie (1963) found that approximately 10% of the colour mutants generated by ethyl methane sulphonate (EMS) and X-ray mutagenesis of *A. thaliana* are variegated. Röbbelen (1968) reported that the *im* variegation mutant (discussed later)

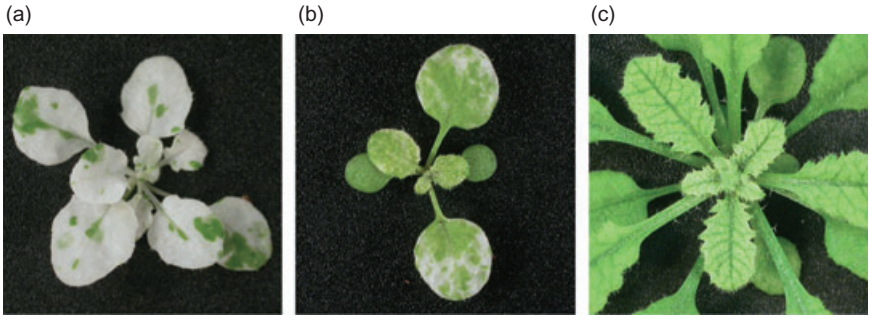

Figure 1. Representative *Arabidopsis* variegation mutants: *immutans* (*im*) (a), *variegated2* (*var2*) (b) and a reticulate mutant deposited at the *Arabidopsis* Biological Resource Center (stock number CS3168) that resembles *chlorophyll a/b-binding protein under-expressed1* (*cue1*) (c).

arose with a frequency of ca. 2×10^{-5} in the M2 progeny from X-ray-treated *A. thaliana* seeds and with a frequency of 1.4×10^{-3} in the M2 progeny from EMS-treated seeds; *im* did not arise spontaneously in any of these experiments. The frequency of EMS-generated *im* in Röbbelen's experiments is higher than the average per locus mutation frequency estimated for EMS mutagenesis in *Arabidopsis* (0.5×10^{-3} to 0.5×10^{-4}) (Koornneef, Dresselhuys & Ramulu 1982; Haughn *et al*. 1988; Vizir, Thorlby & Mulligan 1996). In summary, the *Arabidopsis* mutagenesis experiments, while not exhaustive, are illustrative of the general principle that variegations arise at a low, but variable, frequency in *Arabidopsis*, and that this frequency is influenced by the type of mutagen used.

There are several hundred variegation mutants in the *Arabidopsis* stock centres at Ohio State and Nottingham. Many of these mutants are from the Rédei, Röbbelen and Kranz collections. However, most of these lines have been characterized only superficially and the genes have not been mapped. For instance, a number of uncharacterized lines are alleles of *im* (Wu *et al*. 1999; unpublished observations). Therefore, the diversity of variegations in the stock centres is not clear, but merits further study.

im

The *im* variegation mutant is one of the oldest *Arabidopsis* mutants and was first described and partially characterized nearly 50 years ago by Rédei in the USA and Röbbelen in Germany (Rédei 1963; Röbbelen 1968) (Fig. 1a). Green and white sectoring in *im* is caused by a nuclear recessive gene, and both types of sectors have a uniform (mutant) genetic constitution. The green sectors contain cells with morphologically normal chloroplasts, whereas the white sectors contain vacuolated plastids that lack organized lamellar structures (Wetzel *et al*. 1994). The cells in the white sectors can be heteroplastidic for abnormal plastids and rare, normal-appearing chloroplasts (sometimes called 'mixed cells'). This indicates that the plastids in a given cell do not respond similarly to the *im* mutation (*im* displays 'plastid autonomous' behaviour). Defective plastids are not maternally inherited in *im* and are therefore not permanently defective; likely, the plastid defect is rescued as a consequence of the plastid differentiation events that occur during reproduction and early embryo development (Wetzel *et al*. 1994).

Whereas *im* green sectors contain the normal complement of chlorophylls and carotenoids, the white tissues accumulate phytoene, a non-colored C_{40} carotenoid intermediate (Wetzel *et al*. 1994). This indicates that the mutant is blocked at the phytoene desaturase (PDS) step of carotenoid biosynthesis, and is incapable of producing enough coloured carotenoids to avoid photooxidation; i.e. *im* is a classical carotenoid mutant. Consistent with this idea, green sector formation is enhanced by low light conditions, while high light intensity favours white sector formation. Cloning of *im* by map-based methods (Wu *et al*. 1999) and by T-DNA tagging (Carol *et al*. 1999) revealed that the gene codes for a protein with similarity to the alternative oxidase (AOX) of mitochondrial inner membranes. AOX functions as a terminal oxidase in the alternative (cyanide-resistant) pathway of mitochondrial respiration, where it generates water from ubiquinol (reviewed by Siedow & Umbach 1995; Vanlerberghe & McIntosh 1997). AOX is found in all higher plants and in some algae, fungi and protists. The fact that IM bears similarity to AOX led to the hypothesis that IM is a redox component of a phytoene desaturation pathway involving PDS, plastoquinol and oxygen as a final electron acceptor (reviewed by Aluru *et al*. 2006) (Fig. 2). Consistent with this interpretation, IM has quinol:oxygen oxidoreductase activity when expressed in *Escherichia coli* (Josse *et al*. 2000).

IM is expressed ubiquitously in *Arabidopsis* tissues and organs throughout development (Aluru *et al*. 2001). The well-known *ghost* (*gh*) variegation mutant of tomato is orthologous to *im*, and GH is also abundantly expressed in fruit (Josse *et al*. 2000; Barr *et al*. 2004). In accordance with these observations, the development of many plastid types (chloroplasts, amyloplasts, etioplasts and chromoplasts) is impaired in *im* and *gh* (Aluru *et al*. 2001; Barr *et al*. 2004). The ubiquity of IM expression raises the question whether it plays a role in plastid metabolism that extends beyond carotenoid biosynthesis. Consistent with this idea, it has been proposed that IM serves as the terminal oxidase of chlororespiration [oxidation of plastoquinone (PQ) in the dark]; hence, IM has often been called plastid terminal oxidase (PTOX) (reviewed by Peltier & Cournac 2002). Evidence for this has come from experiments with photosystem I (PSI)-deficient *Chlamydomonas* and with tobacco that over-express *Arabidopsis IM* (Cournac *et al*. 2000; Joet *et al*. 2002). It has also been suggested that IM is a 'safety valve' in photosynthesis, providing an alternate electron

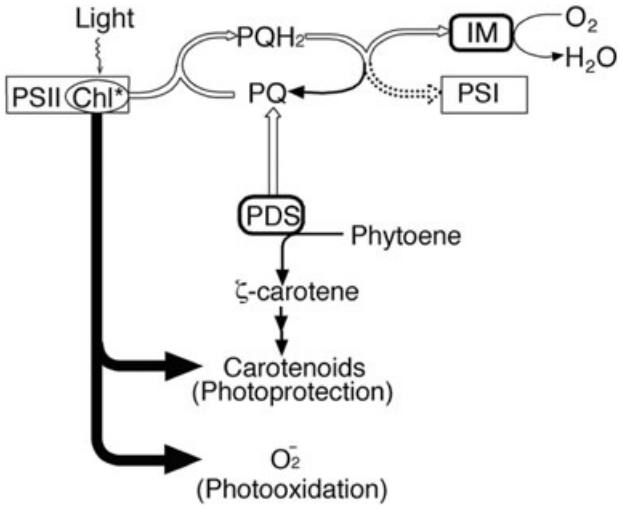

Figure 2. Model of *immutans* (*im*) variegation (adapted from Wu *et al*. 1999). IMMUTANS and other activities facilitate the phytoene desaturation reaction and these activities may explain the mechanism of *im* variegation (see text for details). The concept of compensating activities can also be applied to several other variegation mutants such as *pale cress* (*pac*).

sink to detoxify excess electrons produced during photosynthetic electron transport (as during stress). In support of this idea, IM protein levels are enhanced in double antisense tobacco plants lacking catalase and ascorbate peroxidase, and IM is also induced under high light conditions in WT *Arabidopsis* and tobacco (Rizhsky *et al*. 2002). On the other hand, examination of *im* and *IM* over-expression lines does not support the notion that IM functions as a 'safety valve' during steady-state photosynthesis in *Arabidopsis* (Rosso *et al*. 2006); however, this does not rule out the possibility that IM functions in this capacity during early chloroplast biogenesis. This possibility will be reviewed later (see *Mechanism of im variegation*).

Light microscopy has revealed that the morphology of most organs and tissues is not altered in *im* plants (Aluru *et al*. 2001). However, the green sectors of *im* leaves are thicker than normal because of an increase in air space volume and in epidermal and mesophyll cell sizes. Accompanying these changes are higher-than-normal rates of photosynthesis, an enhanced accumulation of starch and sucrose and altered patterns of partitioning, such that more newly fixed carbon is shunted into the soluble carbohydrate fraction (Aluru *et al*. 2006). These changes appear to be primarily caused by enhanced activities and activation states of key regulatory enzymes, such as ribulose 1·5-bisphosphate carboxylase/oxygenase (Rubisco) and sucrose phosphate synthase.

The anatomy of the white leaf sectors is also perturbed in *im* plants. These sectors have a normal thickness, but the palisade cells fail to expand properly (Aluru *et al*. 2001). This is consistent with the idea that the defective plastids in the white sectors are impaired in the transmission of retrograde signals to regulate leaf development programming (Rodermel 2001). Accompanying the morphological alterations, cells in the white sectors accumulate low levels of sucrose, and have higher cell wall invertase activities than the green sectors. These observations support the notion that there is a sucrose gradient between the green and white sectors (sink demand), and that plant growth and development are optimized by the movement of sucrose from the green tissues (sources) to feed the white tissues (sinks) (Aluru *et al*. 2006).

Mechanism of im variegation

A 'threshold model of photooxidation' has been proposed to explain the mechanism of variegation in null *im* mutants (Wu *et al*. 1999). According to this model, one or more activities are able to compensate for a lack of IM in some plastids and cells of the mutant, allowing the production of chloroplasts, green cells and green sectors. This compensatory activity is unlikely to be another IM-like AOX protein inasmuch as *IM* is a single-copy gene in all organisms examined (it is present only in photosynthetic organisms). However, this activity could be another plastid oxidase involved in oxidation of the PQ pool, e.g. there have been reports of a cyanide-sensitive oxidase in chloroplasts (Joet *et al*. 2002) and of a thylakoid-bound hydroquinone peroxidase (Casano *et al*. 2000), and there could be others (Peltier & Cournac 2002). Alternatively, it is possible that redox components downstream from the PQ pool in the photosynthetic electron transport chain, or other mechanisms of photoprotection (e.g. safety valves) serve as compensating factors, at least during certain stages of thylakoid membrane biogenesis.

According to the working model of *im* variegation (Fig. 2), different pathways of electron transport function in phytoene desaturation at different stages of development, and with different efficiencies, depending on which electron transport components are available (Wu *et al*. 1999). This probably varies from plastid to plastid. A fundamental assumption is that IM is one of these factors and that its presence facilitates carotenoid synthesis during early chloroplast biogenesis when the multisubunit complexes of the thylakoid membrane are being assembled (Mullet 1988; Pyke, Marrison & Leech 1991; Pyke & Leech 1992, 1994). It is proposed that during this time, PDS is unable (or minimally able) to carry out phytoene desaturation when IM is absent and compensating activities (such as downstream electron transport components) are not present. Under these conditions, phytoene would accumulate because of over-reduction of the PQ pool, causing a blockage in carotenoid synthesis; consistent with this hypothesis, an over-reduction of the PQ pool has been observed in *im* green sectors (Baerr *et al*. 2005; Rosso *et al*. 2006). The plastids would thus be in a state vulnerable to high light-induced photooxidation by newly accumulating chlorophylls. In essence, a developmental 'race' would ensue between photooxidation because of a lack of carotenoid photoprotection (giving rise to white plastids) versus the development of efficient mechanisms of electron transport away from phytoene to accommodate PDS activity and the synthesis of

enough carotenoids to afford threshold levels of photoprotection (giving rise to chloroplasts). Low light, and thus, lower photooxidative pressure would allow more plastids to survive the race through the vulnerable stage, accumulate chlorophylls and turn green. In the presence of a functional IM, electron transport would not be inhibited during early development, and carotenoid synthesis would proceed unhindered, thus avoiding photooxidative vulnerability.

A final element of the model proposes that the green and white sectors in developing and mature leaves are primarily a reflection of large-scale differences in restrictive versus permissive conditions perceived by the developing leaf (primarily light). For instance, regions of the meristem that are shaded might translate into green patches in the fully expanded, mature leaf, whereas regions that perceive high light might translate into white patches.

Structure/function studies

IM and AOX are members of the non-heme diiron carboxylate (DOX) protein family, and structural models of these proteins have been proposed based on models of DOX proteins from animal systems (Andersson & Nordlund 1999; Berthold, Andersson & Nordlund 2000). IM and AOX have been modelled as interfacial membrane proteins with an active site (DOX) domain exposed to the stroma (or matrix). The DOX domain is composed of a four-helix bundle that provides six ligands for binding the diiron center: E136, E175, H178, E227, E296 and H299. Using site-directed mutagenesis *in vitro* and *in planta*, Fu, Park & Rodermel (2005) showed that the six Fe ligands of IM are essential for activity and that they do not tolerate change. The mutagenesis experiments also showed that a 16-amino acid domain of IM corresponding to Exon 8 of the genomic sequence (the Exon 8 Domain) is important for function, folding and/or stability. This domain is found in nearly all IM sequences, but lacking in AOX.

var2

yellow variegated was one of the first *Arabidopsis* mutants isolated by Rédei in the 1950s (personal communication). It is allelic to another nuclear recessive mutant, *var2*, isolated by Martínez-Zapater (1993). *var2* has normal-appearing cotyledons and white/yellow sectors in normally green organs of the plant (Martínez-Zapater 1993; Chen, Jensen & Rodermel 1999) (Fig. 1b). The green sectors have morphologically normal chloroplasts, whereas the white and yellow sectors have plastids that appear to be blocked in chloroplast biogenesis inasmuch as they lack an organized thylakoid membrane system (Chen *et al*. 1999; Takechi *et al*. 2000). Some cells in the white sectors of *var2* are heteroplastidic and contain a few normal chloroplasts, in addition to the abnormal plastids (Chen *et al*. 1999). The presence of 'mixed cells' indicates that the mutation has an unequal effect on the plastids in a cell (i.e. that it is 'plastid autonomous'). The extent of *var2* variegation can be modulated by light and development (Martínez-Zapater 1993; Zaltsman, Feder & Adam 2005).

var2 was cloned by map-based procedures and found to encode a chloroplast homolog of *E. coli* FtsH, an ATP-dependent zinc metalloprotease (Chen *et al*. 2000). A T-DNA-tagged allele of VAR2 has also been described (Takechi *et al*. 2000). FtsH belongs to the large class of ATPase associated with various cellular activities (AAA) proteins. AAA proteins have one or two 'AAA cassette' domains (ca. 200–250 amino acids) that contain well-conserved Walker A and B ATP-binding motifs and a 'second region of homology' (function unknown) (Beyer 1997). FtsH proteins contain two transmembrane domains in the N-terminal half of the protein, a single AAA cassette and a zinc-binding motif in the C-terminal half. Localization experiments have shown that VAR2 is embedded in the thylakoid membrane with its bulky C-terminus facing the stroma (Chen *et al*. 2000).

E. coli has a single *FtsH* gene, but *FtsH* genes are present as multigene families in all prokaryotic and eukaryotic photosynthetic organisms examined to date (reviewed by Aluru *et al*. 2006). In *Arabidopsis*, there are 12 *FtsH* genes (Sokolenko *et al*. 2002; Sakamoto *et al*. 2003; Yu, Park & Rodermel 2004). All are located in chloroplasts except AtFtsH3, 4 and 10, which are targeted to mitochondria (Chen *et al*. 2000; Sakamoto *et al*. 2002, 2003; Yu *et al*. 2004). The 12 genes comprise four highly conserved 'phylogenetic pairs' of highly homologous genes (*AtFtsH1/5*, *AtFtsH2/8*, *AtFtsH3/10* and *AtFtsH7/9*) (Sakamoto *et al*. 2003; Yu *et al*. 2004).

Functions of VAR2

The functions of FtsH have been most extensively investigated in *E. coli* where both chaperone and protease activities have been identified (Suzuki *et al*. 1997). In higher plants, it has been established that FtsH is involved in a variety of activities: it degrades unassembled cytochrome b_6f Rieske FeS proteins in thylakoid membranes (Ostersetzer & Adam 1997), it mediates *N*-gene-mediated hypersensitive reactions against the tobacco mosaic virus infection in tobacco (Seo *et al*. 2000), it is involved in phytochrome A-mediated signalling (Tepperman *et al*. 2001) and it might play a role in membrane fusion and/or translocation events because it bears homology to the pepper plastid fusion and/or translocation factor (Pftf) protein (Hugueney *et al*. 1995). Recently, a chloroplast-localized AtFtsH6 protein was found to be responsible for degradation of the light-harvesting complex during high light and senescence (Zelisko *et al*. 2005).

Perhaps the best understood function of chloroplast FtsH is its role in the D1 turnover process (Lindahl *et al*. 2000). The D1 reaction centre protein of photosystem II (PSII) is the target of reactive oxygen species (ROS), and degradation of photodamaged D1 and its replacement by a new copy play a role in protecting the plastid from photoinhibition (Nixon *et al*. 2005). Evidence that FtsH is involved in the D1 turnover process was first reported by Lindahl *et al*. (2000), who showed that degradation of the 23 kDa cleavage product of the photodamaged 32 kDa D1 protein is

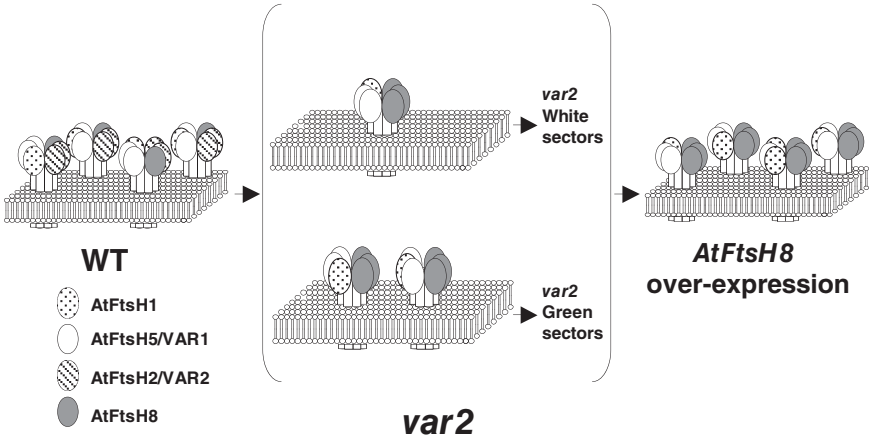

Figure 3. Model for *variegated2* (*var2*) variegation (adapted from Yu *et al*. 2004). See text for details. This model can be expanded to include *variegated1* (*var1*) and several other variegation mutants discussed in the text.

mediated by AtFtsH1. AtFtsH2 and AtFtsH5 might also be involved in D1 turnover inasmuch as *var2* and *variegated1* (*var1*) – an *Arabidopsis* variegation that is caused by a mutation in the nuclear gene for another chloroplast FtsH homolog, AtFtsH5 or VAR1 (Sakamoto *et al*. 2002) – are more prone to PSII photoinhibition, and the D1 degradation process is impaired in *var2* (Bailey *et al*. 2002). An involvement of FtsH in D1 turnover appears to be conserved in both prokaryotic and eukaryotic photosynthetic organisms (Silva *et al*. 2003).

Mechanism of var2 variegation

Functional complementation tests have revealed that AtFtsH2 and AtFtsH8 are functionally interchangeable in *Arabidopsis* chloroplasts, as are AtFtsH1 and AtFtsH5 (Yu *et al*. 2004; Yu, Park & Rodermel 2005). However, members of the 2/8 pair are not interchangeable with members of the 1/5 pair. Members of each pair also have similar expression patterns. Considered together, these observations indicate that the members of each of the two phylogenetic pairs are functionally redundant, at least in part. This conclusion has been supported by double mutant analyses of the various *AtFtsH* genes (Zaltsman, Ori & Adam 2005). On the other hand, the two pairs might have distinct structural or functional roles.

In *E. coli*, FtsH holoenzymes consist of hexamers of a single type of subunit (Akiyama, Yoshihisa & Ito 1995). Two independent efforts have established that FtsH proteases are also present as complexes in thylakoid membranes (Sakamoto *et al*. 2003; Yu *et al*. 2004, 2005). These complexes are composed (at a minimum) of the two phylogenetic pairs – AtFtsH2/8 and AtFtsH1/5; however, their stoichiometry is not known (Sakamoto *et al*. 2003; Yu *et al*. 2004; Zaltsman *et al*. 2005). In support of this idea, the various members of these pairs interact with one another immunologically, and the abundances of the two pairs are mutually dependent on one another inasmuch as all four proteins are decreased coordinately in amount in *var2* or *var1*, and over-expression of AtFtsH8 (in *var2*) or AtFtsH1 (in *var1*) rescues the variegation phenotype by restoring total FtsH pool sizes to normal.

Based on these findings, a threshold model has been proposed to explain the mechanism of *var2* variegation (Yu *et al*. 2004) (Fig. 3). In this model, two pairs of FtsH proteins – AtFtsH1 and 5 and AtFtsH2 and 8 – form oligomeric complexes in the thylakoid membrane and a threshold level of complexes is required for normal chloroplast function and green sector formation. When complex levels fall below the threshold, chloroplast function is impaired and white sectors form, probably as a consequence of photooxidation caused by lack of repair of photodamaged D1 proteins. It was further proposed that proteins within each pair are interchangeable, and that the abundance of proteins in each pair is matched with that of the other pair, with excess subunits being turned over posttranslationally. Thus, over-expression of AtFtsH8 in *var2* serves to stabilize AtFtsH1 and 5.

The idea that variegations arise from the activities of redundant genes plays a dominant theme in the variegations discussed later. It also appears to be a viable explanation for variegations in other species, e.g. *variegated and distorted leaf* (*vdl*) in tobacco (Wang *et al*. 2000). *vdl* codes for a putative DEAD-box RNA helicase that is targeted to chloroplasts.

var2 suppressor screening

To gain a better understanding of FtsH function and the mechanism of *var2* variegation, second-site suppressor screens have been carried out to isolate mutants that modify the *var2* variegation phenotype. One suppressor gene was cloned by map-based methods and found to encode ClpC2, an Hsp100 chaperone that is targeted to the chloroplast stroma (Park & Rodermel 2004). *clpC2* single mutant plants do not have a readily visible phenotype, but they have vastly reduced levels of ClpC2 caused by a splice site mutation in *ClpC2*. *clpC2* and *var2* act antagonistically, and thus it was suggested that VAR2 promotes thylakoid membrane biogenesis while ClpC2 normally serves to inhibit this process, perhaps by enhancing photooxidative stress (directly or indirectly) while the photosynthetic apparatus is being assembled. Further characterization of this mutant and other suppressor lines will lead to a better

understanding of the mechanism of variegation and the regulation of chloroplast development.

var1

The *var1* mutant was isolated as a tissue culture regenerate (Martínez-Zapater 1993). It has normal-appearing cotyledons and green- and white-sectored rosette leaves. The variegation phenotype is expressed in homozygous recessive individuals and the plastid defect is not maternally inherited (the plastids are not permanently defective). The variegation phenotype can be suppressed at growth temperatures lower than ca. 20 °C.

The *VAR1* locus has been cloned and encodes a member of the plastid FtsH metalloprotease gene family (designated VAR1/AtFtsH5) (Sakamoto *et al*. 2002). *var1* mutants are sensitive to photoinhibitory light (Sakamoto *et al*. 2002), and it has been proposed that VAR1 plays an important role in the PSII repair cycle, perhaps as a component of a multimeric FtsH complex (Zaltsman *et al*. 2005). This is consistent with the observation that *var1*/*var2* double mutants have an enhanced variegation phenotype, suggesting that VAR1 and VAR2 act synergistically (Sakamoto *et al*. 2002).

As described earlier, VAR1/AtFtsH5 and AtFtsH1 form a 'phylogenetic pair' of FtsH homologs in *Arabidopsis*, as do VAR2/AtFtsH2 and AtFtsH8 (Yu *et al*. 2005). Overexpression of AtFtsH1, but not VAR2/AtFtsH2, rescues the *var1* phenotype (Yu *et al*. 2005), indicating that VAR1 and AtFtsH1 are functionally redundant, but that VAR2 cannot compensate for VAR1; this could be caused by structural and/or functional constraints. The redundancy of VAR1 and AtFtsH1 suggests that the mechanism of *var1* variegation might be similar to that of *var2* (Fig. 3).

variegated3 (*var3*)

The *var3* mutant was recovered in a stable Ds transposon gene-trapping experiment (Næsted *et al*. 2004). Homozygous recessive plants have green cotyledons and yellow-variegated rosette leaves. Confocal microscopy showed that the *var3* variegation is first visible during early chloroplast biogenesis, shortly before leaf emergence. Palisade cells fail to expand in the yellow sectors, and the plastids in these sectors have only rudimentary lamellae. This supports the idea that the plastid defect in *var3* interrupts the transmission of retrograde signals that specify leaf development.

VAR3 was cloned by taking advantage of the Ds tag (Næsted *et al*. 2004). It encodes an 89.5 kDa protein that contains an N-terminal chloroplast targeting sequence and two zinc fingers (potential protein–protein interaction domains). VAR3 interacts in yeast and *in vitro* with the NCED4 carotenoid dioxygenase, suggesting that the protein might be involved in regulating carotenoid biosynthesis. The *Arabidopsis* genome contains three VAR3 homologs, two of which are predicted to be chloroplast localized. It is therefore possible that the mechanism of *var3* variegation is similar to that of *var2* (Fig. 3).

chloroplast mutator (*chm*)

The *Arabidopsis chm* mutant was first isolated by Rédei (1973) following EMS mutagenesis of *Arabidopsis* seeds. Homozygous recessive individuals have white and yellow sectors in normally green organs of the plant; the variegation trait is inherited in a non-Mendelian fashion. Mesophyll cell differentiation and leaf morphology are also affected in *chm*, leading to a 'rough-leaf' phenotype. Rédei (1973) isolated two *chm* alleles (*chm-1* and *chm-2*) and Martínez-Zapater *et al*. (1992) isolated a third (*chm-3*).

Molecular studies have revealed that the mitochondrial genomes of *chm* plants are rearranged at specific sites, and that these rearrangements cosegregate with the variegation trait (Martínez-Zapater *et al*. 1992). Chloroplast genomes, by contrast, do not appear to be altered in the mutant. Martínez-Zapater *et al*. (1992) suggested that CHM normally prevents the amplification of mutant, subgenomic mitochondrial DNA molecules that are normally present at a low frequency. In the *chm* mutant, these molecules are differentially amplified and cause mitochondrial defects that secondarily result in defective plastids and a variegated phenotype. This suggestion was confirmed by Sakamoto *et al*. (1996) in their studies of the *maternal distorted leaf* (*MDL*) mutant, which was derived from a cross between *chm* and WT plants. They demonstrated that *MDL* had a rearranged mitochondrial genome that had been preferentially amplified at the expense of non-rearranged 'master' (WT) genomes. The rearrangements were likely caused by intragenic ectopic recombination events.

The *CHM* gene has been cloned by map-based methods and found to encode a mitochondrial-targeted protein, designated AtMSH1 (Abdelnoor *et al*. 2003). AtMSH1 shows homology to MutS of *E. coli* and MSH1 of yeast, both of which are involved in DNA mismatch repair and recombination. Although the mechanism is not clear, it is proposed that AtMSH1 controls mitochondrial genome 'substoichiometric shifting' (differential copy number control) either by inhibiting abnormal recombination events or by suppressing the replication of aberrant genomes (Abdelnoor *et al*. 2003). It is assumed that the mechanism of variegation in *chm* is similar to that of the *NCS* mutants of maize, i.e. the permanently defective mitochondria affect chloroplast function secondarily (discussed earlier).

cloroplastos alterados1 (*cla1*)

The *Arabidopsis cla1-1* mutant (altered chloroplasts) is an albino that was isolated by T-DNA tagging (Mandel *et al*. 1996). *CLA1* codes for 1-deoxy-D-xylulose 5-phosphate synthase (DXP synthase), the rate-limiting step of the non-mevalonate, 2-*C*-methyl-D-erythritol-4-P (MEP) pathway of isoprenoid biosynthesis (Estévez *et al*. 2000, 2001). This assignment was verified by biochemical complementation of

the mutant with 1-deoxy-D-xyulose, the product of the DXP synthase reaction (Araki *et al*. 2000; Estévez *et al*. 2000). The MEP pathway is responsible for the synthesis of plastid isoprenoids, including chlorophylls, carotenoids and quinones. The MEP pathway shares intermediates with the mevalonate (MVA) pathway of isoprenoid biosynthesis in the cytosol.

cla1-1 plastids resemble proplastids, having rudimentary thylakoids and an accumulation of vesicles. *CLA1* is expressed throughout the plant, but primarily in young, developing tissues. Given its ubiquity, it is curious that the mutation affects the differentiation of etioplasts and chloroplasts, but not amyloplasts. Nuclear mRNAs for photosynthetic proteins are down-regulated in *cla1-1* tissues, consistent with the notion that nuclear gene transcription is affected by the developmental state of the plastid (retrograde, plastid-to-nucleus signalling). A disruption in retrograde signalling might also be responsible for the observation that *cla1-1* tissues lack palisade cells (Estévez *et al*. 2000).

cla1-1 appears to approximate the null phenotype because *CLA1* mRNAs cannot be detected in the mutant. Two other alleles of *CLA1* have been isolated. The *lovastatin-resistant111* (*lvr111*) mutant was isolated in a screen for lovastatin-resistant root growth (Crowell *et al*. 2003). Lovastatin inhibits 3-hydroxy-3-methylglutaryl coenzyme A (CoA) (HMGCoA) reductase, which catalyses the conversion of HMGCoA to MVA in the cytosolic MVA pathway. *lvr11* mutants are pale green/yellow at the seedling stage and have a variegated dwarf phenotype at the adult stage; the degree of variegation is influenced by light intensity. The *lvr111* gene contains a missense mutation in a non-conserved amino acid of *CLA1* and behaves in a weakly semi-dominant manner (Crowell *et al*. 2003). The third allele of *CLA1*, *chilling sensitive5* (*chs5*), contains a missense mutation in a conserved amino acid that results in a temperature-sensitive phenotype (Araki *et al*. 2000). The mutants appear normal at 22 °C but at 15 °C they develop chlorotic leaves. Temperature-shift experiments using *chs5* revealed that DXP synthase activity is developmentally controlled and required early in chloroplast biogenesis.

The three *CLA1* alleles differ in their severity, with *cla1-1* (putative null, albino) being the most severe and *lvr111* (missense mutation, variegated) the least severe. Although DXP activities have not been measured, phenotypic severity in these mutants correlates with total isoprenoid levels, ranging from 10% of normal for *cla1-1* to 40% of normal for *lvr111* (Crowell *et al*. 2003). Because the white tissues of the various *cla1* mutants lack photoprotective carotenoids, it has been suggested that variegation in *lvr111* is caused by photoinhibition: tissues that receive high amounts of light become photobleached, while those that receive low light are green (Crowell *et al*. 2003). This would be similar to the mechanism of variegation in *im* (Fig. 2). However, it is possible that redundant genes also play a role in the mechanism of variegation inasmuch as Araki *et al*. (2000) reported that *Arabidopsis* contains a gene that bears high homology to *CLA1*.

pale cress (*pac*)

The *Arabidopsis pac* locus is represented by two T-DNA-tagged alleles, designated *pac-1* and *pac-2* (Reiter *et al*. 1994; Grevelding *et al*. 1996). The two alleles differ in their phenotypes: *pac-1* is pale green, contains plastids with rudimentary lamellae and has an altered leaf anatomy with poorly differentiated palisade cells and enlarged epidermal cells (Reiter *et al*. 1994); *pac-2* contains white sectors with abnormal plastids and an altered leaf anatomy, and green leaf sectors with morphologically normal chloroplasts and a normal architecture (Grevelding *et al*. 1996). The *PAC* gene product is a light-regulated chloroplast protein (Reiter *et al*. 1994; Tirlapur *et al*. 1999) that might play a role in plastid mRNA processing (Meurer *et al*. 1998). *Lhcb* expression is not down-regulated in the pale green or white *pac* leaf sectors, suggesting that loss of PAC does not trigger the retrograde signalling pathway that normally down-regulates the expression of nuclear genes for chloroplast proteins (Reiter *et al*. 1994). On the other hand, plastid-to-nucleus developmental signalling appears to be interrupted in the mutant inasmuch as leaf architecture is perturbed (Rodermel 2001).

The reason for the differences in phenotype between *pac-1* and *pac-2* is unclear (both are T-DNA-tagged, putative nulls), but might be related to leaky expression or to the different ecotypes in which the mutants were isolated. There appears to be one *PAC* gene in the *Arabidopsis* genome (Yu & Rodermel, unpublished data), suggesting that redundant gene expression cannot account for the differences between *pac-1* and *pac-2*, or for the mechanism of variegation in *pac-2*. However, it is interesting that *pac* can be rescued by feeding with cytokinin (Grevelding *et al*. 1996). This suggests that the mechanism of variegation might involve compensation by redundant pathways or processes in a plastid autonomous manner, similar to the general mechanism proposed for *im* (Fig. 2).

white cotyledons (*wco*)

The nuclear recessive *wco* mutant was isolated from a T-DNA insertion population, but the gene is not linked to the insert and *WCO* has not been cloned (Yamamoto, Puente & Deng 2000). Cotyledons of *wco* are albino, whereas the true leaves are green. The green tissues have normal-appearing chloroplasts, but plastids in the white tissues contain plastoglobuli and have rudimentary thylakoids; they do not resemble white, photobleached plastids. Transcripts from nuclear genes for photosynthetic components are present at WT levels in the white *wco* cotyledons, but they are decreased in these tissues following treatment with norflurazon. This suggests that the retrograde signalling pathway that controls the expression of nuclear genes for photosynthetic proteins is able to function in the white cells, but only after photooxidation by norflurazon (i.e. the pathway fails to be initiated in the mutant). In contrast to nuclear mRNAs, most plastid mRNAs are reduced in abundance in *wco* cotyledons. An exception is 16S pre-rRNAs,

which accumulate. This accumulation has led to the proposal that the primary defect in *wco* resides in cotyledon-specific 16S rRNA maturation (Yamamoto *et al*. 2000).

thf1

Korth and colleagues identified an *Arabidopsis* ortholog of a potato, nuclear light- regulated gene which they called *THYLAKOID FORMATION1* (*THF1*) (Wang *et al*. 2004). They found that *THF1* is targeted to plastids, that it is light regulated and that its sequence is conserved among oxygenic photoautotrophs. Knockout (T-DNA insertion) *thf1* mutants are severely stunted and variegated, while antisense lines have varying degrees of growth inhibition and variegation, depending on the line and illumination conditions. Some of the antisense lines grow slowly under short day conditions, but resemble WT in size and coloration at bolting. This is consistent with the idea that growth factors are able to compensate for a lack of THF1.

The name, *THF1*, comes from the observation that plastids in the white/yellow sectors of *thf1* mutants accumulate vesicles and lack organized lamellar structures, suggesting that they are defective in thylakoid membrane biogenesis (Wang *et al*. 2004); thylakoid development is thought to occur by transport and fusion of vesicles from the inner envelope membrane early in the chloroplast biogenesis process (Westphal, Soll & Vothknecht 2003). However, Pakrasi and colleagues found that THF1 is orthologous to Psb29 in *Synechocystis* sp. PCC6803, that THF1 cofractionates with PSII preparations in *Arabidopsis* and that the *thf1* mutant is impaired in PSII function (Keren *et al*. 2005). They suggested that THF1 regulates PSII biogenesis. A third group (Huang *et al*. 2006) reported that THF1 is ubiquitously expressed in *Arabidopsis* tissues and organs, and that it is localized in the stroma, the outer plastid membrane, and in stromules, which are tube-like extensions of the plastid that have been implicated in mediating intracellular signalling (Kwok & Hanson 2004). Huang *et al*. (2006) also found that THF1 interacts with GPA1 (the Gα subunit of the plasma membrane G-protein heterotrimer) at sites of stromule/plasma membrane interaction. They proposed that THF1 might play a role in G-protein-linked D-glucose sugar signalling. Clearly, the primary lesion in *thf1* needs to be clarified before the mechanism of variegation can be addressed.

atase2 deficient (*atd2*)

The *atd2* mutant was isolated in a T-DNA insertion population, and the tag was used to clone *ATD2* (van der Graaff 1997; León *et al*. 1998). *ATD2* codes for glutamine 5-phosphoribosylpyrophosphate amidotransferase [also called amidophosphoribosyl transferase (ATase)], which is the first committed step of 'de novo' purine synthesis. The identity of *ATD2* was verified by functional complementation of *atd2* with a WT ATase gene, as well as by chemical complementation with inosine.

atd2 has green cotyledons, albino true leaves and a reduced stature that is caused by (in part) the absence of palisade cells and an underdeveloped vasculature. Mutants of the same gene in tobacco are similarly stunted with chlorotic leaves (van der Graaff *et al*. 2004). Chloroplasts in the cotyledons of *atd2* appear normal, while plastids in cells of the white leaves are vesiculated. This suggests that *atd2* is disrupted in the transmission of a plastid signal that regulates palisade cell differentiation.

There are two ATase isozymes in *Arabidopsis*, ATase1 and ATase2; *atd2* codes for ATase2. The two genes are differentially expressed, with ATase1 being expressed primarily in flowers and roots and ATase2 in true leaves. Differential expression of these genes might explain the pattern of variegation in *atd2*, similar to the mechanism proposed for *var2* (Fig. 3).

albomaculans (*am*)

The *am* mutant is one of the oldest *Arabidopsis* variegations, and was generated by X-ray treatment of pollen (Röbbelen 1966; Hagemann 1986). Variegation is induced in homozygous recessive plants. Whereas *am* is inherited in a Mendelian fashion, the variegation trait is inherited maternally, suggesting that *am* generates permanently defective plastids (and/or mitochondria). 'Mixed (heteroplastidic) cells' are present in the white sectors; these cells have morphologically normal chloroplasts, as well as abnormal, non-pigmented plastids that are vesiculated and contain plastoglobules. The *AM* gene has not been cloned.

Reticulate mutants: *differential development of vascular-associated cells1* (*dov1*), *reticulata* (*re*) *lower cell density1-1* (*lcd1-1*), *scabra3* (*sca3*), *chlorophyll a/b-binding protein under-expressed1* (*cue1*)

A large number of reticulate ('net-like') mutants are available at the Nottingham and Ohio State Stock Centers or in other mutant collections (reviewed by González-Bayón *et al*. 2006). Few of these have been characterized at the molecular level. Reticulate mutants typically have a green (or dark green) vasculature on a pale green/yellow lamina, or alternatively, a pale vasculature on a green lamina. This patterning presumably reflects differences in mesophyll (M) versus bundle sheath (BS) cells that surround the vasculature. In all reported cases, the reticulate pattern is inherited in a nuclear recessive manner, and the cells of the plant have a uniform genetic constitution. Four of the best-characterized reticulate mutants will be discussed here: *dov1* (Kinsman & Pyke 1998), *re* (Rédei & Hirono 1964; Barth & Conklin 2003; González-Bayón *et al*. 2006), *sca3* (Hricová, Quesada & Micol 2006) and *cue1* (Li *et al*. 1995; López-Juez *et al*. 1998; Streatfield *et al*. 1999, Knappe *et al*. 2003; Voll *et al*. 2003).

dov1 leaves have a green vasculature and pale green/yellow interveinal regions (Kinsman & Pyke 1998). BS cells

have normal-appearing chloroplasts, whereas plastids in the M cells are reduced in size and number, lack grana and are vacuolated; some M cells are heteroplastidic and contain rare, normal-appearing chloroplasts. Mature *dov1* plants are small but flower normally. The fact that leaf anatomy and plastid morphology are disrupted in the interveinal regions of *dov1* indicates that the mutant is perturbed in the transmission of plastid-to-nucleus developmental signals. *DOV1* has not been cloned, but it has been suggested that the gene product functions to control events during early chloroplast biogenesis in M cells.

The cotyledons, vegetative leaves and cauline leaves of *re* mutants have dark green paraveinal regions and pale green interveinal regions (Rédei & Hirono 1964). *RE* is ubiquitously expressed, but organs other than leaves are not affected by the mutation. González-Bayón and coworkers have recently isolated 7 *re* alleles, four of which are putative nulls (González-Bayón *et al*. 2006). Chloroplast number and function are not impaired in the M cells, but the interveinal regions are pale because of decreased M cell densities (Barth & Conklin 2003; González-Bayón *et al*. 2006). Leaf size and shape are not perturbed in the various *re* alleles, and the lack of M cells is compensated for by an increase in air space volume.

RE has been positionally cloned (González-Bayón *et al*. 2006) and is allelic to *LCD1*, a gene that encodes a chloroplast-targeted, 47 kDa transmembrane protein of unknown function (Barth & Conklin 2003). *LCD1-1* was initially of interest because a mutant allele, *lcd1-1*, has an ozone- and *Pseudomona*s-sensitive phenotype; it is also susceptible to oxidative stress generated in the apoplast (Barth & Conklin 2003). Despite the diversity of phenotypes associated with *re* (*lcd1-1*), it has been concluded that the primary defect in the mutant involves the decrease in M cell density, and was proposed that RE might function in early leaf primordia development to control M cell division. Interestingly, double mutant analyses revealed that *RE* and *CUE1* (discussed later) act in a leaf developmental pathway separate from one that involves *DOV1*. The mechanism of variegation in *re* (and *lcd1-1*) is unclear, but there are several genes in the *Arabidopsis* genome with high similarity to *RE* (Barth & Conklin 2003). An operating hypothesis is that redundant RE activities compensate for a lack of RE in the normal-appearing tissues of the mutant.

The *SCABROUS3* gene codes for RpoTp, a nuclear-encoded, plastid-targeted RNA polymerase (Hricová *et al*. 2006). There are three *sca3* alleles, the most severe of which is reticulated with a pale green/yellow lamina and green vasculature. Cells in the vasculature appear normal but the interveinal regions have an impaired anatomy and a decreased M cell density. Plastid size, number and morphology are abnormal in the M cells. These observations are consistent with the idea that M cell proliferation and differentiation are controlled, in part, by plastid-to-nucleus developmental signals (Rodermel 2001). Hricová *et al*. (2006) suggested that the primary lesion in *sca3* is a defect in RpoTp-mediated transcription of plastid genes required for the conversion of proplastids to chloroplasts. They also proposed that the pattern of variegation in *sca3* is caused by the action of a redundant activity, RpoTmp, in the green and non-green tissues of the mutant. RpoTmp is a nuclear-encoded RNA polymerase that is dually targeted to mitochondria and plastids.

The *cue* mutants were identified in a genetic screen for positively acting components of light signalling pathways in *Arabidopsis* (Li *et al*. 1995; López-Juez *et al*. 1998). *cue1* is the most extensively studied of these, and it exhibits a reticulate phenotype (pale green interveinal regions and dark green paraveinal regions). The vasculature has normal-appearing BS cells, but the M cells are reduced in number and have an altered morphology. Chloroplast size, but not number, is reduced in the M cells. Several other *cue* mutants appear to have a similar phenotype, at least superficially (López-Juez *et al*. 1998).

CUE1 encodes a plastid inner envelope phosphoenolpyruvate/phosphate translocator (PPT) that imports phosphoenolpyruvate (PEP) into the stroma (Streatfield *et al*. 1999). PEP is involved in the biosynthesis of fatty acids, amino acids and isoprenoids, and is the first substrate of the shikimate pathway, which produces aromatic amino acids and a variety of secondary metabolites (Knappe *et al*. 2003). Consistent with the idea that flux into the shikimate pathway is impaired in *cue1*, the pale green sectors of the mutant have lower than normal concentrations of metabolites derived from this pathway, and the mutant phenotype can be rescued by feeding aromatic amino acids (Streatfield *et al*. 1999). *cue1* can also be rescued by over-expresssion of either a heterologous PPT or a C4-type pyruvate, orthophosphate dikinase (PPDK) (Voll *et al*. 2003).

There are two *PPT* genes in the *Arabidopsis* genome (*CUE1*/*AtPPT1* and *AtPPT2*) and both are targeted to the plastid (Knappe *et al*. 2003). The two genes are redundant (at least in part) because over-expression of *AtPPT2* can partially complement *cue1* (Knappe *et al*. 2003). *AtPPT1* is expressed primarily in the vasculature whereas *AtPPT2* expression predominates in interveinal regions; however, there is overlap in expression of the two genes, providing an explanation for the reticulate phenotype of *cue1* (Knappe *et al*. 2003).

The fact that both leaf and plastid anatomy are altered in *cue1* suggests that the mutant is defective in plastid-to-nucleus developmental signals that specify mesophyll development (Rodermel 2001; Knappe *et al*. 2003). Consistent with this notion, Knappe *et al*. (2003) proposed that AtPPT1 is involved in the generation of phenylpropanoid metabolism-derived signal molecules that trigger development in the interveinal regions. By contrast, other retrograde signalling pathways might be operational in *cue1* inasmuch as transcripts from nuclear genes encoding chloroplast proteins are under-expressed in the mutant.

In summary, the interveinal regions of reticulate mutants have impaired leaf anatomies with fewer than normal mesophyll cells; in some mutants, M cell morphology is also perturbed. Alterations in leaf anatomy are frequently, but not always, accompanied by a defect in plastid number, size and/or state of differentiation, indicating that the mutant is

impaired in the transmission of retrograde signals that affect leaf developmental programming. In nearly all cases of reticulate mutants, it can be hypothesized that the mechanism of variegation involves the operation of redundant genes.

CONCLUSIONS

One advantage of variegations is that they provide a means of studying genes for proteins that are important for chloroplast development, but for which mutant analysis is difficult, either because mutations in a gene-of-interest are lethal (e.g. albinos) or because they do not show a readily distinguishable phenotype, perhaps because the defect is buffered by a compensating activity or a redundant gene product. Variegations arise at a low, but variable, frequency in mutagenesis experiments, and in cases where the green and white sectors have the same (mutant) genotype, much can be learned about the function of the gene product by studying its mode of action in both tissue types, and by deciphering the mechanism of variegation in the mutant plant. With the current interest in understanding the functions of all the genes in the *Arabidopsis* genome, ca. 3500 of which are believed to be nuclear genes for chloroplast proteins (Peltier *et al*. 2002), the isolation of more variegations (and suppressors of variegation) will be an important tool for understanding the functions of these genes, especially 'unknowns'. For instance, suppressors of *var2* variegation, such as ClpC2, can be viewed as 'reporters' for gene products that play a role in the processes of photoinhibition and/or chloroplast biogenesis. This provides a point of entry for understanding the function of the gene-of-interest in a focused, detailed fashion.

This paper has considered a number of nuclear gene-induced variegations, primarily in *Arabidopsis*, and it has focused on *im* and *var2* as representing two variegation mechanism paradigms. However, both of these mechanisms place emphasis on the notion of 'plastid autonomy' and the hypothesis that plastids have intrinsic differences in substrate amounts and in rates of reactions involved in fundamental plastid processes. Both mechanisms also suggest that these inequalities play a role in determining whether a given plastid is able to attain a threshold level of an activity that is essential for normal chloroplast development. It is proposed that these inequalities are important especially early in chloroplast biogenesis, when the components of the photosynthetic apparatus are synthesized and assembled, and that these inequalities are manifested as sectors in the mature leaf. In the case of *var2*, it was hypothesized that redundant gene products are a way to influence the amount of substrate needed to attain threshold levels of D1 repair activity, and thus to form a normal chloroplast. This type of mechanism appears to be applicable to many of the variegations discussed earlier, although hard evidence is lacking. Yet, redundant gene activities cannot be invoked for *im* or for some of the other variegations for which redundant genes have not been identified.

The notion of plastid autonomy has been emphasized as playing a crucial role in the genesis of 'chaotic variegations', in which sectoring in the mature leaf follows no discernible pattern. However, superimposed on plastid autonomy is a 'cell autonomous' mechanism of variegation, readily apparent in the reticulate mutants. These mutants have a defined pattern of variegation that corresponds to cell and/or tissue type, e.g. paraveinal regions (BS cells) are green and interveinal regions (M cells) are yellow. These sorts of variegation are likely caused by cell-specific expression of gene family members that masks underlying differences among plastids.

REFERENCES

Abdelnoor R.V., Yule R., Elo A., Christensen A.C., Meyer-Gauen G. & Mackenzie S.A. (2003) Substoichiometric shifting in the plant mitochondrial genome is influenced by a gene homologous to muts. *Proceedings of the National Academy of Sciences of the USA* **100,** 5968–5973.

Akiyama Y., Yoshihisa T. & Ito K. (1995) FtsH, a membrane-bound ATPase, forms a complex in the cytoplasmic membrane of *Escherichia coli*. *Journal of Biological Chemistry* **270,** 23485–23490.

Aluru M., Bae H., Wu D. & Rodermel S. (2001) The *Arabidopsis immutans* mutation affects plastid differentiation and the morphogenesis of white and green sectors in variegated plants. *Plant Physiology* **127,** 67–77.

Aluru M., Yu F., Fu A. & Rodermel S. (2006) *Arabidopsis* variegation mutants: new insights into chloroplast biogenesis. *Journal of Experimental Botany* **57,** 1871–1881.

Andersson M.E. & Nordlund P. (1999) A revised model of the active site of alternative oxidase. *FEBS Letters* **449,** 17–22.

Araki N., Kusumi K., Masamoto K., Niwa Y. & Iba K. (2000) Temperature-sensitive *Arabidopsis* mutant defective in 1-deoxy-D-xylulose 5-phosphate synthase within the plastid nonmevalonate pathway of isoprenoid biosynthesis. *Physiologia Plantarum* **108,** 19–24.

Baerr J.N., Thomas J.D., Taylor B.G., Rodermel S.R. & Gray G.R. (2005) Differential photosynthetic compensatory mechanisms exist in the *immutans* mutant of *Arabidopsis thaliana*. *Physiologia Plantarum* **124,** 390–402.

Bailey S., Thompson E., Nixon P.J., Horton P., Mullineaux C.W., Robinson C. & Mann N.H. (2002) A critical role for the Var2 FtsH homologue of *Arabidopsis thaliana* in the photosystem II repair cycle *in vivo*. *Journal of Biological Chemistry* **277,** 2006–2011.

Barr J., White W.S., Chen L., Bae H. & Rodermel S. (2004) The GHOST terminal oxidase regulates developmental programming in tomato fruit. *Plant, Cell & Environment* **27,** 840–852.

Barth C. & Conklin P.L. (2003) The lower cell density of leaf parenchyma in the *Arabidopsis thaliana* mutant *lcd1-1* is associated with increased sensitivity to ozone and virulent *Pseudomonas syringae*. *Plant Journal* **35,** 206–218.

Bellaoui M., Keddie J.S. & Gruissem W. (2003) DCL is a plant-specific protein required for plastid ribosomal RNA processing and embryo development. *Plant Molecular Biology* **53,** 531–543.

Berthold D.A., Andersson M.E. & Nordlund P. (2000) New insight into the structure and function of alternative oxidase. *Biochimica et Biophysica Acta* **1460,** 241–254.

Beyer A. (1997) Sequence analysis of the AAA protein family. *Protein Science* **6,** 2043–2058.

Bogorad L. (1981) Chloroplasts. *Journal of Cell Biology* **91,** 256s–270s.

Bonnema A.B., Castillo C., Reiter N., Cunningham M., Adams H.P. & O'Connell M. (1995) Molecular and ultrastructural analysis of a nonchromosomal variegated mutant. Tomato mitochondrial mutants that cause abnormal leaf development. *Plant Physiology* **109,** 385–392.

Bonnett H.T., Djurberg I., Fajardo M. & Glimelius K. (1993) A mutation causing variegation and abnormal development in tobacco is associated with an altered mitochondrial DNA. *Plant Journal* **3,** 519–525.

Börner T. & Sears B.B. (1986) Plastome mutants. *Plant Molecular Biology Reporter* **4,** 69–92.

Brutnell T.P., Sawers R.J.H., Mant A. & Langdale J.A. (1999) BUNDLE SHEATH DEFECTIVE2, a novel protein required for post-translational regulation of the *rbcL* gene of maize. *Plant Cell* **11,** 849–864.

Carol P., Stevenson D., Bisanz C., Breitenbach J., Sandmann G., Mache R., Coupland G. & Kuntz M. (1999) Mutations in the *Arabidopsis* gene *IMMUTANS* cause a variegated phenotype by inactivating a chloroplast terminal oxidase associated with phytoene desaturation. *Plant Cell* **11,** 57–68.

Casano L.M., Zapata J.M., Martin M. & Sabater B. (2000) Chlororespiration and poising of cyclic electron transport. *Journal of Biological Chemistry* **275,** 942–948.

Chang T.-L., Stoike L.L., Zarka D., Schewe G., Chiu W.-L., Jarrell D.C. & Sears B.B. (1996) Characterization of primary lesions caused by the plastome mutator of *Oenothera*. *Current Genetics* **30,** 522–530.

Chatterjee M. & Martin C. (1997) Tam3 produces a suppressible allele of the *DAG* locus of *Antirrhinum majus* similar to Mu-suppressible alleles of maize. *Plant Journal* **11,** 759–771.

Chatterjee M., Sparvoli S., Edmunds C., Garosi P., Findlay K. & Martin C. (1996) *DAG*, a gene required for chloroplast differentiation and palisade development in *Antirrhinum majus*. *EMBO Journal* **15,** 4194–4207.

Chen M., Jensen M. & Rodermel S.R. (1999) The *yellow variegated* mutant of *Arabidopsis* is plastid autonomous and delayed in chloroplast biogenesis. *Journal of Heredity* **90,** 207–214.

Chen M., Choi Y., Voytas D.F. & Rodermel S.R. (2000) Mutations in the *Arabidopsis VAR2* locus cause leaf variegation due to the loss of a chloroplast FtsH protease. *Plant Journal* **22,** 303–313.

Coe E.H., Thompson D. & Walbot V. (1988) Phenotypes mediated by the *iojap* genotype in maize. *American Journal of Botany* **75,** 634–644.

Connett M.B. (1987) Mechanisms of maternal inheritance of plastids and mitochondria: developmental and ultrastructural evidence. *Plant Molecular Biology Reporter* **4,** 193–205.

Cournac L., Redding K., Ravenel J., Rumeau D., Josse E., Kuntz M. & Peltier G. (2000) Electron flow between photosystem II and oxygen in chloroplasts of photosystem I-deficient algae is mediated by a quinol oxidase involved in chlororespiration. *Journal of Biological Chemistry* **275,** 17256–17262.

Crowell D.N., Packard C.E., Pierson C.A., Giner J.-L., Downes B.P. & Chary S.N. (2003) Identification of an allele of *CLA1* associated with variegation in *Arabidopsis thaliana*. *Physiologia Plantarum* **118,** 29–37.

Estévez J.M., Cantero A., Romero C., Kawaide H., Jiménez L.F., Kuzuyama T., Seto H., Kamiya Y. & León P. (2000) Analysis of the expression of *CLA1*, a gene that encodes the 1-deoxyxylulose 5-phosphate synthase of the 2-C-methyl-D-erythritol-4-phosphate pathway in Arabidopsis. *Plant Physiology* **124,** 95–104.

Estévez J.M., Cantero A., Reindl A., Reichler S. & León P. (2001) 1-deoxy-D-xylulose 5-phosphate synthase, a limiting enzyme for plastidic isoprenoid biosynthesis in plants. *Journal of Biological Chemistry* **276,** 22901–22909.

Federoff N.V. (1989) Maize transposable elements. In *Mobile DNA* (eds D.E. Berg & M.M. Howe), pp. 375–411. American Society for Microbiology, Washington, D.C.

Feschotte C., Jiang N. & Wessler S.R. (2002) Plant transposable elements: where genetics meets genomics. *Nature Reviews Genetics* **3,** 329–341.

Fu A., Park S. & Rodermel S. (2005) Sequences required for the activity of PTOX (IMMUTANS), a plastid terminal oxidase: *in vitro* and *in planta* mutagenesis of iron-binding sites and a conserved sequence that corresponds to exon 8. *Journal of Biological Chemistry* **280,** 42489–42496.

Gabay-Laughnan S. & Newton K.J. (2005) Mitochondrial mutations in maize. *Maydica* **50,** 349–359.

Glick R.E. & Sears B.B. (1994) Genetically programmed chloroplast dedifferentiation as a consequence of plastome–genome incompatibility in Oenothera. *Plant Physiology* **106,** 367–373.

Goldschmidt-Clermont M. (1998) Coordination of nuclear and chloroplast gene expression in plant cells. *International Review of Cytology* **177,** 115–180.

González-Bayón R., Kinsman E.A., Quesada V., Vera A., Robles P., Ponce M.R., Pyke K.A. & Micol J.L. (2006) Mutations in the *RETICULATA* gene dramatically alter internal architecture but have little effect on overall organ shape in *Arabidopsis* leaves. *Journal of Experimental Botany* **57,** 3019–3031.

van der Graaff E. (1997) *Developmental mutants of* Arabidopsis thaliana *obtained after T-DNA transformation*. PhD thesis, Leiden University, Leiden, the Netherlands.

van der Graaff E., Hooykaas P., Lein W., Lerchl J., Kunze G., Sonnewald U. & Boldt R. (2004) Molecular analysis of 'de novo' purine biosynthesis in solanaceous species and in *Arabidopsis thaliana*. *Frontiers in Bioscience* **9,** 1803–1816.

Granick, S. (1955). Die plastiden und chondriosomen. In *Encyclopedia of Plant Physiology* (ed W. Ruhland) Volume **1**, pp. 507–564. Springer Verlag, Berlin, Germany.

Grevelding C., Suter-Crazzolara C., von Menges A., Kemper E., Masterson R., Schell J. & Reiss B. (1996) Characterization of a new allele of *pale cress* and its role in greening in *Arabidopsis thaliana*. *Molecular & General Genetics* **251,** 532–541.

GuhaMajumdar M., Baldwin S. & Sears B.B. (2004) Chloroplast mutations induced by 9-aminoacridine hydrochloride are independent of the *plastome mutator* in *Oenothera*. *Theoretical Applied Genetics* **108,** 543–549.

Gutierres S., Sabar M., Lelandais C., Chetrit P., Diolez P., Degand H., Boutry M., Vedel F., De Kouchkovsky Y. & De Paepe R. (1997) Lack of mitochondrial and nuclear-encoded subunits of complex I and alteration of the respiratory chain in *Nicotiana sylvestris* mitochondrial deletion mutants. *Proceedings of the National Academy of Sciences of the USA* **94,** 3436–3441.

Hagemann R. (1986) A special type of nucleus–plastid-interactions: nuclear gene induced plastome mutants. In *Regulation of Chloroplast Differentiation* (eds G. Akoyunoglou & H. Senger), pp. 455–466. Alan R. Liss, New York, NY, USA.

Hall L.N., Rossini L., Cribb L. & Langdale J.A. (1998) GOLDEN 2: a novel transcriptional regulator of cellular differentiation in the maize leaf. *Plant Cell* **10,** 925–936.

Han C.-D. & Martienssen R.A. (1995) The iojap protein (IJ) is associated with 50S chloroplast ribosomal subunits. *Maize Genetics Cooperative Newsletter* **69,** 32.

Han C.-D., Coe E.H. & Martienssen R.A. (1992) Molecular cloning and characterization of *iojap* (*ij*), a pattern striping gene of maize. *EMBO Journal* **11,** 4037–4046.

Haughn G.W., Smith J., Mazur B. & Somerville C. (1988) Transformation with a mutant *Arabidopsis acetolactate synthase* gene renders tobacco resistant to sulfonylurea herbicides. *Molecular & General Genetics* **211,** 266–271.

Hess W.R., Müller A., Nagy F. & Börner T. (1994) Ribosome-deficient plastids affect transcription of light-induced nuclear

genes: genetic evidence for a plastid-derived signal. *Molecular & General Genetics* **242,** 305–312.

Hricová A., Quesada V. & Micol J.-L. (2006) The *SCABRA3* nuclear gene encodes the plastid RpoTp RNA polymerase, which is required for chloroplast biogenesis and mesophyll cell proliferation in Arabidopsis. *Plant Physiology* **141,** 942–956.

Hsieh M.-H. & Goodman H.M. (2005) The *Arabidopsis* IspH homolog is involved in the plastid nonmevalonate pathway of isoprenoid biosynthesis. *Plant Physiology* **138,** 641–653.

Huang J., Taylor J.P., Chen J.-G., Uhrig J.F., Schnell D.J., Nakagawa T., Korth K.L. & Jones A.M. (2006) The plastid protein THYLAKOID FORMATION1 and the plasma membrane G-Protein GPA1 interact in a novel sugar-signaling mechanism in Arabidopsis. *Plant Cell* **18,** 1226–1238.

Hudson A., Carpenter R., Doyle S. & Coen E.S. (1993) *Olive*: a key gene required for chlorophyll biosynthesis in *Antirrhinum majus*. *EMBO Journal* **12,** 3711–3719.

Hugueney P., Bouvier F., Badillo A., D'Harlingue A., Kuntz M. & Camara B. (1995) Identification of a plastid protein involved in vesicle fusion and/or membrane protein translocation. *Proceedings of the National Academy of Sciences of the USA* **92,** 5630–5634.

Joet T., Genty B., Josse E.M., Kuntz M., Cournac L. & Peltier G. (2002) Involvement of a plastid terminal oxidase in plastoquinone oxidation as evidenced by expression of the *Arabidopsis thaliana* enzyme in tobacco. *Journal of Biological Chemistry* **277,** 31623–31630.

Josse E.M., Simkin A.J., Gaffe J., Labourne A., Kuntz M. & Carol P. (2000) A plastid terminal oxidase associated with carotenoid desaturation during chromoplast differentiation. *Plant Physiology* **123,** 1427–1436.

Keddie J.S., Carroll B., Jones J.D.G. & Gruissem W. (1996) The *DCL* gene of tomato is required for chloroplast development and palisade cell morphogenesis in leaves. *EMBO Journal* **15,** 4208–4217.

Keren N., Ohkawa H., Welsh E.A., Liberton M. & Pakrasi H.B. (2005) Psb29, a conserved 22-kD protein, functions in the biogenesis of photosystem II complexes in *Synechocystis* and Arabidopsis. *Plant Cell* **17,** 2768–2781.

Kinsman E.A. & Pyke K.A. (1998) Bundle sheath cells and cell-specific plastid development in *Arabidopsis* leaves. *Development* **125,** 1815–1822.

Kirk J.T.O. & Tilney-Bassett R.A.E. (1978) *The Plastids*, 2nd edn. Elsevier/North-Holland, Amsterdam, the Netherlands.

Klimyuk V.I., Nussaume L., Harrison K. & Jones J.D.G. (1995) Novel GUS expression patterns following transposition of an enhancer trap Ds element in Arabidopsis. *Molecular & General Genetics* **249,** 357–365.

Knappe S., Löttgert T., Schneider A., Voll L., Flügge U.-I. & Fischer K. (2003) Characterization of two functional *phosphoenolpyruvate/phosphate translocator* (PPT) genes in *Arabidopsis – AtPPT1* may be involved in the provision of signals for correct mesophyll development. *Plant Journal* **36,** 411–420.

Koornneef M., Dresselhuys H.C. & Ramulu K.S. (1982) The genetic identification of translocations in Arabidopsis. *Arabidopsis Information Service* **19,** 93–99.

van der Krol A.R., Lenting P.E., Veenstra J., Van Der Meer I.M., Koes R.E., Gerats A.G.M., Mol J.N.M. & Stuitje A.R. (1988) An antisense *chalcone synthase* gene in transgenic plants inhibits flower pigmentation. *Nature* **333,** 866–869.

van der Krol A.R., Mur L.A., de Lange P., Gerats A.G.M., Mol J.N.M. & Stuitje A.R. (1990) Antisense *chalcone synthase* genes in petunia: visualization of variable transgene expression. *Molecular & General Genetics* **220,** 204–212.

Kuromori T., Wada T., Kamiya A. et al. (2006) A trial of phenome analysis using 4000 *Ds*-insertional mutants in gene-coding regions of *Arabidopsis*. *Plant Journal* **47,** 640–651.

Kwok E.Y. & Hanson M.R. (2004) Stromules and the dynamic nature of plastid morphology. *Journal of Microscopy* **214,** 124–137.

Langdale J.A. & Kidner C.A. (1994) Bundle sheath defective, a mutation that disrupts cellular differentiation in maize leaves. *Development* **120,** 673–681.

León P., Arroyo A. & Mackenzie S. (1998) Nuclear control of plastid and mitochondrial development in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **49,** 453–480.

Li H., Culligan K., Dixon R.A. & Chory J. (1995) CUE1: a mesophyll cell-specific positive regulator of light-controlled gene expression in Arabidopsis. *Plant Cell* **7,** 1599–1610.

Lindahl M., Spetea C., Hundal T., Oppenheim A.B., Adam Z. & Andersson B. (2000) The thylakoid FtsH protease plays a role in the light-induced turnover of the photosystem II D1 protein. *Plant Cell* **12,** 419–431.

López-Juez E., Jarvis R.P., Takeuchi A., Page A.M. & Chory J. (1998) New *Arabidopsis cue* mutants suggest a close connection between plastid- and phytochrome regulation of nuclear gene expression. *Plant Physiology* **118,** 803–815.

Maliga P. (2004) Plastid transformation in higher plants. *Annual Review of Plant Biology* **55,** 289–313.

Mandel M.A., Feldmann K.A., Herrera-Estrella L., Rocha-Sosa M. & León P. (1996) *CLA1*, a novel gene required for chloroplast development, is highly conserved in evolution. *Plant Journal* **9,** 649–658.

Martienssen R.A., Barkan A., Freeling M. & Taylor W.C. (1989) Molecular cloning of a maize gene involved in photosynthetic membrane organization that is regulated by Robertson's Mutator. *EMBO Journal* **8,** 1633–1639.

Martínez-Zapater J.M. (1993) Genetic analysis of variegated mutants in Arabidopsis. *Journal of Heredity* **84,** 138–140.

Martínez-Zapater J.M., Gil P., Capel J. & Somerville C.R. (1992) Mutations at the *Arabidopsis CHM* locus promote rearrangements of the mitochondrial genome. *Plant Cell* **4,** 889–899.

McKelvie A.D. (1963) Studies in the induction of mutations in *Arabidopsis thaliana* (L.) Heynh. *Radiation Botany* **3,** 105–123.

Meins F., Si-Ammour A. & Blevins T. (2005) RNA silencing systems and their relevance to plant development. *Annual Review of Cell and Developmental Biology* **21,** 297–318.

Meurer J., Grevelding C., Westhoff P. & Reiss B. (1998) The PAC protein affects the maturation of specific chloroplast mRNAs in *Arabidopsis thaliana*. *Molecular & General Genetics* **258,** 342–351.

Mullet J.E. (1988) Chloroplast development and gene expression. *Annual Review of Plant Physiology and Plant Molecular Biology* **39,** 475–502.

Næsted H., Holm A., Jenkins T. et al. (2004) *Arabidopsis VARIEGATED 3* encodes a chloroplast-targeted, zinc-finger protein required for chloroplast and palisade cell development. *Journal of Cell Science* **117,** 4807–4818.

Newton K. & Coe E.J. (1986) Mitochondrial DNA changes in abnormal growth mutants of maize. *Proceedings of the National Academy of Sciences of the USA* **83,** 7363–7366.

Newton K.J., Knudsen C., Gabay-Laughnan S. & Laughnan J.R. (1990) An abnormal growth mutant in maize has a defective mitochondrial cytochrome oxidase gene. *Plant Cell* **2,** 107–113.

Nixon P.J., Barker M., Boehm M., de Vries R. & Komenda J. (2005) FtsH-mediated repair of the photosystem II complex in response to light stress. *Journal of Experimental Botany* **56,** 357–363.

Nott A., Jung H.-S., Koussevitzky S. & Chory J. (2006) Plastid-to-nucleus retrograde signaling. *Annual Review of Plant Biology* **57,** 739–759.

Ostersetzer O. & Adam Z. (1997) Light-stimulated degradation of an unassembled Rieske FeS protein by a thylakoid-bound protease: the possible role of the FtsH protease. *Plant Cell* **9,** 957–965.

Park S. & Rodermel S.R. (2004) Mutations in ClpC2/Hsp100 suppress the requirement for FtsH in thylakoid membrane biogenesis. *Proceedings of the National Academy of Sciences of the USA* **101,** 12765–12770.

Park S.H., Park S.H., Chin H.G., Cho M.J., Martienssen R.A. & Han C. (2000) Inhibitor of striate conditionally suppresses cell proliferation in variegated maize. *Genes & Development* **14,** 1005–1016.

Peltier G. & Cournac L. (2002) Chlororespiration. *Annual Review of Plant Biology* **53,** 523–550.

Peltier J., Emanuelsson O., Kalume D.E., Ytterberg J., Frisco G., Rudella A., Liberies D.A., Soderberg L., Roepstorff P. & von Heijne G. (2002) Central functions of the lumenal and peripheral thylakoid proteome of *Arabidopsis* determined by experimentation and genome-wide prediction. *Plant Cell* **14,** 211–236.

Pfannschmidt T. (2003) Chloroplast redox signals: how photosynthesis controls its own genes. *Trends in Plant Science* **8,** 33–41.

Pyke K.A. & Leech R.M. (1992) Chloroplast division and expansion is radically altered by nuclear mutations in *Arabidopsis thaliana*. *Plant Physiology* **99,** 1005–1008.

Pyke K.A. & Leech R.M. (1994) A genetic analysis of chloroplast division and expansion in *Arabidopsis thaliana*. *Plant Physiology* **104,** 201–207.

Pyke K.A., Marrison J.L. & Leech R.M. (1991) Temporal and spatial development of the cells of the expanding first leaf of *Arabidopsis thaliana* (L.) Heynh. *Journal of Experimental Botany* **42,** 1407–1416.

Raghavendra A.S., Padmasree K. & Saradadevi K. (1994) Interdependence of photosynthesis and respiration in plant cells: interactions between chloroplasts and mitochondria. *Plant Science* **97,** 1–14.

Rédei G.P. (1963) Somatic instability caused by a cysteine-sensitive gene in Arabidopsis. *Science* **139,** 767–769.

Rédei G.P. (1973) Extra-chromosomal mutability determined by a nuclear gene locus in Arabidopsis. *Mutation Research* **18,** 149–162.

Rédei G.P. & Hirono Y. (1964) Linkage studies. *Arabidopsis Information Service* **1,** 9–10.

Reiter R.S., Coomber S.A., Bourett T.M., Bartley G.E. & Scolnik P.A. (1994) Control of leaf and chloroplast development by the *Arabidopsis* gene pale cress. *Plant Cell* **6,** 1253–1264.

Rizhsky L., Hallak-Herr E., Van Breusegem F., Rachmilevitch S., Barr J.E., Rodermel S., Inzé D. & Mittler R. (2002) Double antisense plants lacking ascorbate peroxidase and catalase are less sensitive to oxidative stress than single antisense plants lacking ascorbate peroxidase or catalase. *Plant Journal* **32,** 329–342.

Röbbelen G. (1966) Chloroplastendifferenzierung nach geninduzierter Plastommutation bei *Arabidopsis thaliana* (L.) Heynh. *Zeitschrift für Pflanzenphysiologie* **55,** 387–403.

Röbbelen G. (1968) Genbedingte Rotlicht-Empfindlichkeit der Chloroplastendifferenzierung bei Arabidopsis. *Planta* **80,** 237–254.

Rodermel S.R. (2001) Pathways of plastid-to-nucleus signaling. *Trends in Plant Science* **6,** 471–478.

Rosso D., Ivanov A.G., Fu A. et al. (2006) IMMUTANS does not act as a stress-induced safety valve in the protection of the photosynthetic apparatus of *Arabidopsis thaliana* during steady state photosynthesis. *Plant Physiology* **142,** 574–585.

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

Sakamoto W., Tamura T., Hanba-Tomita Y., Sodmergen & Murata M. (2002) The *VAR1* locus of *Arabidopsis* encodes a chloroplastic FtsH and is responsible for leaf variegation in the mutant alleles. *Genes to Cells* **7,** 769–780.

Sakamoto W., Zaltsman A., Adam Z. & Takahashi Y. (2003) Coordinated regulation and complex formation of *yellow variegated 1* and *yellow variegated 2*, chloroplastic FtsH metalloproteases involved in the repair cycle of photosystem II in *Arabidopsis* thylakoid membranes. *Plant Cell* **15,** 2843–2855.

Schultes N.P., Brutnell T.P., Allen A., Dellaporta S.L., Nelson T. & Chen J. (1996) *Leaf permease1* gene of maize is required for chloroplast development. *Plant Cell* **8,** 463–475.

Seo S., Okamoto M., Iwai T., Iwano M., Fukui K., Isogai A., Nakajima N. & Ohashi Y. (2000) Reduced levels of chloroplast FtsH protein in tobacco mosaic virus-infected tobacco leaves accelerate the hypersensitive reaction. *Plant Cell* **12,** 917–932.

Settles A.M., Baron A., Barkan A. & Martienssen R.A. (2001) Duplication and suppression of chloroplast protein translocation genes in maize. *Genetics* **157,** 349–360.

Siedow J.N. & Umbach A.L. (1995) Plant mitochondrial electron transfer and molecular biology. *Plant Cell* **7,** 821–831.

Silva P., Thompson E., Bailey S., Kruse O., Mullineaux C.W., Robinson C., Mann N.H. & Nixon P.J. (2003) FtsH is involved in the early stages of repair of photosystem II in *Synechocystis* sp. PCC 6803. *Plant Cell* **15,** 2152–2164.

Sokolenko A., Pojidaeva E., Zinchenko V., Panichkin V., Glaser V.M., Herrmann R.G. & Shestakov S.V. (2002) The gene complement for proteolysis in the cyanobacterium *Synechocystis* sp. PCC 6803 and *Arabidopsis thaliana* chloroplasts. *Current Genetics* **41,** 291–310.

Stoike L.L. & Sears B.B. (1998) Plastome mutator-induced alterations arise in *Oenothera* chloroplast DNA through template slippage. *Genetics* **149,** 347–353.

Streatfield S.J., Weber A., Kinsman E.A., Häusler R.E., Li J., Post-Beittenmiller D., Kaiser W.M., Pyke K.A., Flügge U. & Chory J. (1999) The phosphoenolpyruvate/phosphate translocator is required for phenolic metabolism, palisade cell development, and plastid-dependent nuclear gene expression. *Plant Cell* **11,** 1609–1621.

Stubbe W. (1989) *Oenothera* – an ideal system for studying the interactions of genome and plastome. *Plant Molecular Biology Reporter* **7,** 245–257.

Suzuki C.K., Rep M., van Dijl J.M., Suda K., Grivell L.A. & Schatz G. (1997) ATP-dependent proteases that also chaperone protein biogenesis. *Trends in Biochemical Sciences* **22,** 118–123.

Takechi K. Sodmergen, Murata M., Motoyoshi F. & Sakamoto W. (2000) The *YELLOW VARIEGATED* (*VAR2*) locus encodes a homologue of FtsH, an ATP-dependent protease in Arabidopsis. *Plant & Cell Physiology* **41,** 1334–1346.

Tepperman J.M., Zhu T., Chang H.-S. & Quail P.H. (2001) Multiple transcription-factor genes are early targets of phytochrome A signaling. *Proceedings of the National Academy of Sciences of the USA* **98,** 9437–9442.

Tilney-Bassett R.A.E. (1975) Genetics of variegated plants. In *Genetics and Biogenesis of Mitochondria and Chloroplasts* (eds C.W. Birky, P.S. Perlman & T.J. Byers), pp. 268–308. Ohio State University Press, Columbus, OH, USA.

Tilney-Bassett R.A.E. (1986) *Plant Chimeras*. Edward Arnold Publishers Ltd, London, England.

Tilney-Bassett R.A.E. (1989) The diversity of the structure and function of higher plant plastids. In *Physiology, Biochemistry, and Genetics of Nongreen Plastids* (eds C.D. Boyer, J.C. Shannon & R.C. Hardison), pp. 1–14. American Society of Plant Physiologists, Rockville, MD, USA.

Tirlapur U.K., Dahse I., Reiss B., Meurer J. & Oelmüller R. (1999) Characterization of the activity of a plastid-targeted green fluo

rescent protein in Arabidopsis. *European Journal of Cell Biology* **78,** 233–240.

Tsugane K., Maekawa M., Takagi K., Takahara H., Qian Q., Eun C.-H. & Iida S. (2006) An active DNA transposon *nDart* causing leaf variegation and mutable dwarfism and its related elements in rice. *Plant Journal* **45,** 46–57.

Vanlerberghe G.C. & McIntosh L. (1997) Alternative oxidase: from gene to function. *Annual Review of Plant Physiology and Plant Molecular Biology* **48,** 703–734.

Vizir I., Thorlby G. & Mulligan B. (1996) Classical mutagenesis and genetic analysis. In *Plant Gene Isolation: Principles and Practice* (eds G.D. Foster & D. Twell), pp. 216–245. John Wiley & Sons Ltd, New York, NY, USA.

Voll L., Häusler R.E., Hecker R., Weber A., Weissenböck G., Fiene G., Waffenschmidt S. & Flügge U.I. (2003) The phenotype of the *Arabidopsis cue1* mutant is not simply caused by a general restriction of the shikimate pathway. *Plant Journal* **36,** 301–317.

Walbot V. & Coe E.H. (1979) Nuclear gene *iojap* conditions a programmed change to ribosome-less plastids in *Zea mays*. *Proceedings of the National Academy of Sciences of the USA* **76,** 2760–2764.

Wang Q., Sullivan R.W., Kight A., Henry R.L., Huang J., Jones A.M. & Korth K.L. (2004) Deletion of the chloroplast-localized *Thylakoid Formation1* gene product in *Arabidopsis* leads to deficient thylakoid formation and variegated leaves. *Plant Physiology* **136,** 3594–3604.

Wang Y.-C., Duby G., Purnelle B. & Boutry M. (2000) Tobacco *VDL* gene encodes a plastid DEAD box RNA helicase and is involved in chloroplast differentiation and plant morphogenesis. *Plant Cell* **12,** 2129–2142.

Westphal S., Soll J. & Vothknecht U.C. (2003) Evolution of chloroplast vesicle transport. *Plant & Cell Physiology* **44,** 217–222.

Wetzel C.M., Jiang C., Meehan L.J., Voytas D.F. & Rodermel S.R. (1994) Nuclear-organelle interactions: the *immutans* variegation mutant of *Arabidopsis* is plastid autonomous and impaired in carotenoid biosynthesis. *Plant Journal* **6,** 161–175.

Wisman E., Hartmann U., Sagasser M., Baumann E., Palme K., Hahlbrock K., Saedler H. & Weisshaar B. (1998) Knock-out mutants from an En-1 mutagenized *Arabidopsis thaliana* population generate phenylpropanoid biosynthesis phenotypes. *Proceedings of the National Academy of Sciences of the USA* **95,** 12432–12437.

Wu D., Wright D.A., Wetzel C., Voytas D.F. & Rodermel S. (1999) The *IMMUTANS* variegation locus of *Arabidopsis* defines a mitochondrial alternative oxidase homolog that functions during early chloroplast biogenesis. *Plant Cell* **11,** 43–55.

Yamamoto Y.Y., Puente P. & Deng X.-W. (2000) An *Arabidopsis* cotyledon-specific albino locus: a possible role in 16S rRNA maturation. *Plant & Cell Physiology* **41,** 68–76.

Yaronskaya E., Ziemann V., Walter G., Averina N., Börner T. & Grimm B. (2003) Metabolic control of the tetrapyrrole biosynthetic pathway for porphyrin distribution in the barley mutant albostrians. *Plant Journal* **35,** 512–522.

Yu F., Park S. & Rodermel S.R. (2004) The *Arabidopsis FtsH metalloprotease* gene family: interchangeability of subunits in chloroplast oligomeric complexes. *Plant Journal* **37,** 864–876.

Yu F., Park S. & Rodermel S.R. (2005) Functional redundancy of AtFtsH metalloproteases in thylakoid membrane complexes. *Plant Physiology* **138,** 1957–1966.

Zaltsman A., Feder A. & Adam Z. (2005) Developmental and light effects on the accumulation of FtsH protease in *Arabidopsis* chloroplasts – implications for thylakoid formation and photosystem II maintenance. *Plant Journal* **42,** 609–617.

Zaltsman A., Ori N. & Adam Z. (2005) Two types of FtsH protease subunits are required for chloroplast biogenesis and photosystem II repair in Arabidopsis. *Plant Cell* **17,** 2782–2790.

Zelisko A., García-Lorenzo M., Jackowski G., Jansson S. & Funk C. (2005) AtFtsH6 is involved in the degradation of the light-harvesting complex II during high-light acclimation and senescence. *Proceedings of the National Academy of Sciences of the USA* **102,** 13699–13704.

Received 16 October 2006; received in revised form 3 November 2006; accepted for publication 13 November 2006

