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An *Arabidopsis* Cotyledon-Specific Albino Locus: a Possible Role in 16S rRNA Maturation

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We report here the isolation and characterization of a cotyledon-specific albino locus of *Arabidopsis, WHITE COTYLEDONS (WCO)*. This recessive mutation in the *WCO* locus, located on the top of Chromosome 1, results in albino cotyledons but green true leaves. An accumulation profile of chlorophylls and ultrastructure of chloroplasts indicate that *WCO* is necessary for development of functional chloroplasts in cotyledons but is dispensable in true leaves. This was further supported by the fact that the mutants request feeding of sucrose for their survival at the early seedling stage where true leaves have not emerged, but the mutants which have developed true leaves are able to grow autotrophically without sucrose supplementation. The *wco* mutants accumulate low levels of chloroplast mRNA encoding photosynthesis-related proteins and have a specific defect in 16S rRNA maturation in a cotyledon-specific manner. Although *wco* mutants exhibited abnormal chloroplasts and chloroplast gene expression in cotyledons, nuclear genes for photosynthetic components are expressed at similar levels to those found in wild-type siblings. This lack of suppression of the nuclear genes is not due to a defect in the signaling of the so-called ''plastid factor'' to the nucleus since normal suppression of the nuclear genes was observed in response to the photo-oxidative damage due to norflurazon application.

Key words: 16S rRNA maturation — Albino — *Arabidopsis* — Chloroplast gene expression — Chloroplast structure — Plastid signal.

Chloroplasts have their own genome which encodes a small proportion of the components required for their function. The chloroplast genes and their expression machinery share many features of those shown by prokaryotic cells (Gruissem and Tonkyn 1993, Sugiura 1992). However, some eukaryotic features, such as extensive RNA processing and poly (A) addition to mRNAs have also been reported (Gruissem and Tonkyn 1993, Kudla et al. 1996, Lisitsky et al. 1996, Sugiura 1992). Furthermore, chloroplast RNA undergoes many specific types of metabolism such as trans-splicing and RNA editing (Sugiura 1992). Therefore, chloroplasts have developed their own complex system to regulate gene expression in response to environmental signals and their developmental state (Gruissem and Tonkyn 1993, Mayfield et al. 1995, Stern et al. 1997).

The chloroplast components are not all encoded by the chloroplast genome, some being encoded by the nuclear genome. Therefore, chloroplast biogenesis requires the coordinated expression of nuclear and chloroplast genes. In higher plants, chloroplast development is strictly light-dependent. As expected, many nuclear-encoded genes for photosynthesis-related proteins, such as genes for chlorophyll *a/b*-binding proteins (*CAB*) and the small subunit of ribulose 1,5-bisphosphate carboxylase (*RbcS*), are expressed at high levels upon exposure to light (Gilmartin et al. 1990, Kuhlemeier et al. 1987, Thompson and White 1991, Tobin and Kehoe 1994). For most of those photosynthesis-related genes, their ability to respond to light signals is also thought to be under the control of a so-called ''plastid factor'' reflecting plastid development (Oelmüller 1989, Taylor 1989). This hypothetical signaling pathway is thought to mediate the state of the chloroplasts and accordingly up- or down-regulate the nuclear genes for photosynthetic components. This signaling was proposed on the base of several experimental systems, including etioplast-to-chloroplast transition, developing chloroplasts, chloroplasts with defective ribosomes, photodamaged chloroplasts, and defective chloroplasts due to the malfunction of metabolic pathways (Hess et al. 1994, Mandel et al. 1996, Oelmüller 1989, Kruse et al. 1995, Taylor 1989, Tonkyn et al. 1992). However, it is not clear if all these reports reflect the same signaling pathway, because in most cases, only the state of chloroplasts and the response of the nuclear gene expression as an output are reported without any consensus of the connecting signaling pathways.

It has been established that in dicotyledonous plants the cotyledons, embryonic leaves formed during embryogenesis, are distinct in their developmental characteristics from true leaves, which are the result of apical meristem

Abbreviations: GUS, β-glucuronidase; MU, 4-methylumbelliferone; MUG, 4-methylumbelliferyl β-D-glucuronide; NF, norflurazon; SSLP, simple sequence length polymorphisms.

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activity. These two types of leaves are separate and have distinct developmental processes. Several *Arabidopsis* mutations have been identified which transform true leaves into cotyledons (Conway and Poethig 1997). Other *Arabidopsis* mutations have also been defined which change the identity of cotyledons into true leaves (Parcy et al. 1997, Lotan et al. 1998). Although both cotyledons and true leaves contain chloroplasts and are photosynthetically active, their chloroplast development follows different paths (Mansfield and Briarty 1996). In the cotyledons, the plastids partially initiate development during embryogenesis to reach some intermediate state but stop during seed maturation and dormancy. Upon germination in the light, the plastids further develop into functional chloroplasts. During the development of the normal true leaves, proplastids are differentiated into young chloroplasts, and finally mature chloroplasts. The final form of chloroplasts in the cotyledons normally contains a less extensive thylakoid membrane compared to the mature chloroplasts in true leaves and is more similar to young chloroplasts of true leaves (Deng and Gruissem 1987).

In this report, we describe the *Arabidopsis WHITE COTYLEDONS (WCO)* locus, mutations of which display cotyledon-specific albino phenotype. Molecular analysis revealed that the *wco* mutants have severe reduction of chloroplast gene expression with the full expression of nuclear-encoded genes for photosynthetic components, demonstrating uncoupling of chloroplast development and the nuclear gene expression. Furthermore, analysis of chloroplast RNA species revealed that *wco* has a defect in 16S rRNA maturation in a cotyledon-specific manner, which is suggested to be the primarily lesion that leads to the developmental defect of the chloroplast.

Materials and Methods

Plant growth and documentation—All the *Arabidopsis* lines used in this work were in the No-0 ecotype except for the mapping studies. Unless otherwise mentioned, *Arabidopsis* seeds were surface sterilized and plated on GM medium (Valvekens et al. 1988) supplemented with 1% (w/v) sucrose and 0.8% (w/v) Bactoagar (Difco, Detroit, MI, U.S.A.). After 2–5 d of vernalization, seeds were germinated and grown at 22°C under white light with a long day photoperiod (16 h light/8 h dark). Exposure to light more than 100 $\mu E\ m^{-2}\ s^{-1}$ was avoided throughout the life cycle of *wco* plants. In order to rescue *wco* seedlings, the young mutant seedlings were transferred to a new agar medium and laid down so as to give good contact between the hypocotyl and the agar surface. Seedlings which developed several green leaves were transferred to soil and grown to maturity. Seedlings were examined and photographed with a dissection microscope, and the image was saved on slide film, incorporated into a Macintosh computer with a slide scanner, processed with Photoshop software (Adobe Systems, San Jose, CA, U.S.A.), and printed using a digital printer (Pictrography 2000; Fuji Photo Film U.S.A., Elmsford, NY, U.S.A.).

Examination of chloroplast structure with a transmission electron microscope has been described previously (Puente et al. 1996).

Chlorophyll quantitation—For chlorophyll quantitation, seedlings of wild-type or *wco* (Lines #9 and #19) were grown on GM with 3% (w/v) sucrose and 0.8% (w/v) Bactagar at 22°C. Harvested tissue was frozen at −80°C and subsequently subjected to chlorophyll quantitation according to a published method (Arnon 1949). No significant differences were observed between different mutant lines and thus only the result of one line (#9) is shown here.

Genetic analysis—For complementation tests, 10 independently isolated mutant lines including the lines #9, #19, and #28 were randomly selected and their putative heterozygotes ($WCO^{+/-}$) were inter-crossed by pollination. The genotype of the parental plants used for the crosses was revealed by observation of their selfed progeny. In general, 8 to 80 F_1 seeds per cross were analyzed for the seedling and adult phenotype. Representative F_1 plants were selfed and their F_2 progeny were analyzed to confirm the F_1 result.

For chromosome mapping, two independent mutants (#9 and #28) in the No-0 ecotype were crossed with Col-0 plants carrying the *gl1* mutation, and homozygote *wco* seedlings from the F_2 population were subjected to PCR-based SSLP analysis (Bell and Ecker 1994). The map position of *wco* was located to the ''south'' of nga63 based on the following observations. Among the four identified recombinants between *wco* and nga63 (homozygous at *wco* (No-0/No-0) and heterozygous at nga63 (No-0/Col)) from the 306 F_2 mutants, one recombinant was heterozygous at ATEAT1 and homozygous for No-0 at ATSRP54A and AtZFPG (see Fig. 2 for the map position of the markers. AtZFPG is ''south'' of ATSRP54A). Three other recombinants were also homozygous for No-0 at ATSRP54A.

Analysis of CAB1::GUS expression—A representative *wco* mutant (#19) was crossed into an established CAB1::GUS homozygote line also in the No-0 ecotype (Deng et al. 1991), and double homozygote lines were subsequently obtained ($WCO^{-/-}$; $CAB1::GUS^{+/+}$) for GUS expression analysis. Seeds of the wild-type and *wco* mutants carrying homozygous promoter-GUS were plated on GM medium with 1% sucrose, 0.8% Bactoagar, 0.1% (v/v) ethanol which was used as a solvent of norflurazon (NF; SAN-9789, Sandoz Pharmaceutical, East Hanover, NJ, U.S.A.), and with or without 100 nM NF (Puente et al. 1996). Seeds on the medium were germinated and grown at 22°C under fluorescent light (16 h light/8 h dark) at a light intensity of 80 $\mu E\ m^{-2}\ s^{-1}$. The 7-day-old seedlings were subjected to GUS expression analyses. Activity staining and quantitative assay of GUS was carried out according to Puente et al. (1996), and Yamamoto et al. (1997), respectively.

RNA analysis—Seedlings were grown on GM media supplemented with 0.8% agar and 1% sucrose under a light regime of 16 h light/8 h dark. Total RNA was extracted from the seedlings at the middle of the light period according to Yamamoto et al. (1995). Equal amounts of total RNA were subjected to Northern analyses (Yamamoto et al. 1998) using ^{32}P-labeled probes of *CAB, RbcS, psbA, rbcL*, 18S rDNA, and 16S rDNA (Deng et al. 1991). Radioactivity of each hybridizing band was quantified using a Fujix BAS2000 (Fuji Photo Film U.S.A.).

Results

Identification of the WHITE COTYLEDON locus—During previous root explant transformations (Puente et al. 1996), 25 apparently independently derived transgenic

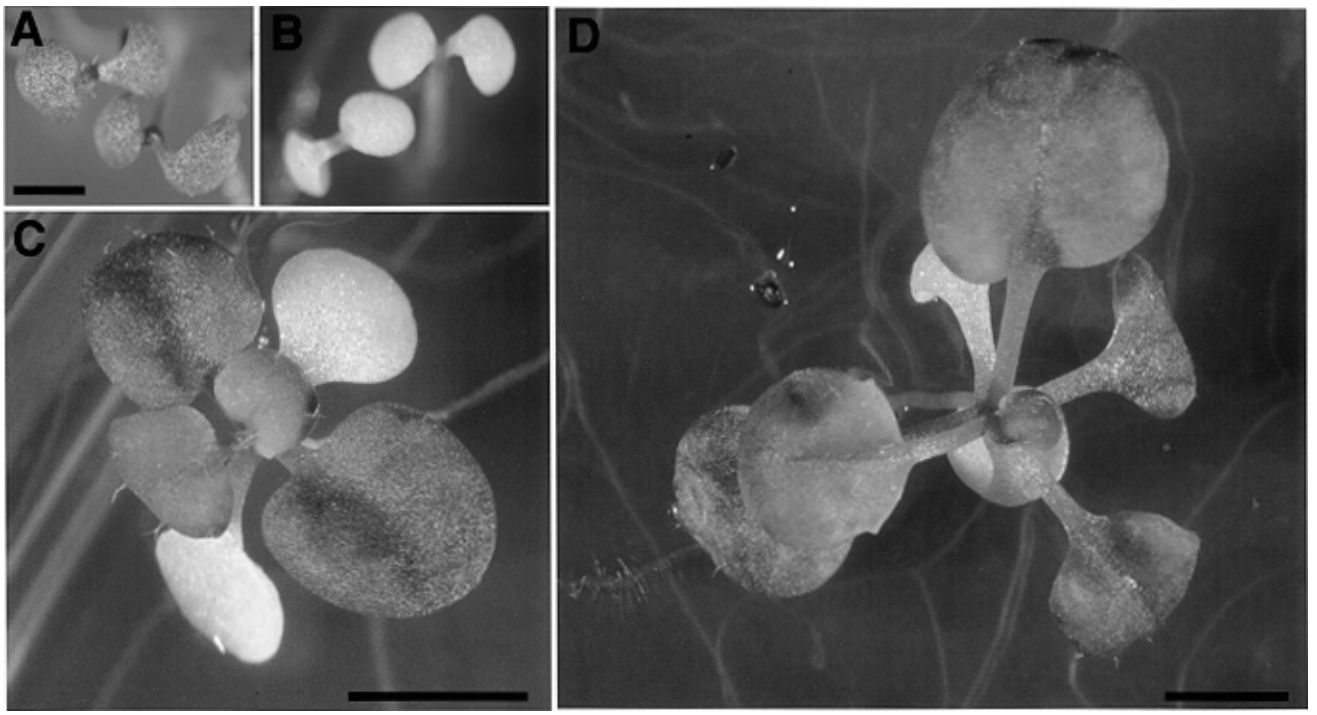

Fig. 1 The visible phenotype of the *wco* mutants at different developmental stages. (A) Cotyledons of 7-day-old wild-type seedlings. (B) Cotyledons of 7-day-old mutant seedlings. (C) A 2-week-old mutant seedling; and (D) A 3-week-old mutant seedling. The bar in (A) indicates 1 mm and applies to both (A) and (B). Bars in (C) and (D) indicate 2 mm. Note that the cotyledons are the first pair of white leaves at the bottom in (C) and (D).

lines segregated about a quarter of the same cotyledon-specific albino phenotype in their progeny (Fig. 1). When those mutants were crossed with wild-type, F_1 plants showed a wild-type phenotype and one quarter of the F_2 plants displayed the cotyledon-specific albino phenotype, suggesting that the mutations were genetically stable and recessive. Genetic complementation tests with ten randomly picked mutant lines suggested that all the mutations are allelic and belong to a single complementation group (data not shown). Therefore, we have concluded that most, if not all the mutants, define a single genetic locus.

To further characterize this locus, the fine map position of *wco* was determined with two mutant lines. Both mutations were mapped to the same location between nga63 and ATSRP54A on Chromosome 1 (Fig. 2). Since there are no other known albino mutants which map in this area of the chromosome, it is concluded that these mutations define a novel genetic locus, designated as *WHITE COTYLEDONS (WCO)*. Because the *wco* locus is not linked to the T-DNA in the transgenic lines (data not shown), the mutants were outcrossed and mutant lines without T-DNA were established and used for further analyses.

Chlorophyll accumulation in cotyledons and leaves—As shown in Figure 1, 7-day-old *wco* mutants showed the albino phenotype with drastically diminished chlorophyll content in the cotyledons. However, true leaves, which emerged later, were green and healthy. Therefore, the *wco* mutants, unlike any previously described albino mutants, are defective primarily in the cotyledons. To assess the degree of the defect in chloroplast development in cotyledons and true leaves, chlorophyll accumulation of these tissue was quantified (Table 1). The 7-day-old mutant seedling accumulated as little as 3% of the chlorophylls seen

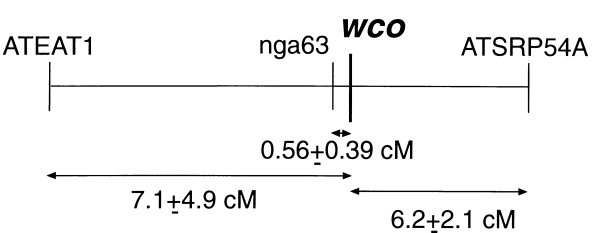

Fig. 2 The *WCO* locus maps to Chromosome 1 of the *Arabidopsis* genome. Only a small region of the top arm of Chromosome 1 is shown. The right side of diagram is toward the centromere and the left side is toward the telomere. The observed recombination crossovers are: 4 in 56 chromosomes between ATEAT1 and *wco*; 4 in 712 chromosomes between nga63 and *wco*; and 16 in 260 chromosomes between *wco* and ATSRP54A. For further details, see Materials and Methods.

Table 1 Chlorophyll accumulation in the aerial parts of the wild-type and *wco* mutants

	17 μE m^{-2} s^{-1}			150 μE m^{-2} s^{-1}		
	mg Chl($a+b$) /g FW [a]	Relative amount	Chla/b ratio	mg Chl($a+b$) /g FW [a]	Relative amount	Chla/b ratio
WT 7-day-old [b]	1,110 ± 66	100%	2.62±0.09	738.2 ± 56.1	100%	2.74±0.04
wco 7-day-old [b]	63.79± 1.36	5.75%	2.54±0.04	22.80± 2.61	3.09%	3.50±0.04
WT 3-week-old [c]	1,433 ±336	100%	2.84±0.05	1,779 ±230.0	100%	2.98±0.32
wco 3-week-old [c]	989.7 ± 92.8	69.1%	2.30±0.05	421.4 ±111.6	23.7%	2.17±0.05

[a] Averages±standard deviations of chlorophyll (Chl) concentrations for 4 independent measurements. FW: fresh weight excluding roots.
[b] represents chlorophyll accumulation in the cotyledons.
[c] represents chlorophyll accumulation in the true leaves.

in the wild-type seedlings under a standard light condition for growth (150 μE m^{-2} s^{-1}). Reduction of light intensity to 17 μE m^{-2} s^{-1} did not drastically change the chlorophyll accumulation relative to wild-type (5.75%), indicating that the phenotype is not light intensity-dependent. In both light conditions, the chlorophyll a/b ratio of *wco* was not extremely higher or lower than that of wild-type, indicating that *wco* does not specifically lack either chlorophyll *a* or *b*. In 3-week-old mutant plants which consist of mostly true leaves (Fig. 1D), chlorophyll content was about 23.7% compared to that of the wild-type under the standard light conditions (150 μE m^{-2} s^{-1}). Low irradiance (17 μE m^{-2} s^{-1}) resulted in recovery of chlorophyll accumulation in the mutant to about 69% of the wild-type level (Table 1). Thus, although the defect in chlorophyll accumulation appears to be cotyledon-specific (Fig. 1), true leaves also accumulate reduced amount of chlorophylls, especially at high irradiance.

The wco mutants have a severely impaired chloroplast ultrastructure—The effect of *wco* mutations on chloroplast structure in cotyledons was examined by transmission electron microscopy (Fig. 3). As controls, wild-type chloroplasts (Fig. 3A) and photobleached chloroplasts (Fig. 3B) caused by feeding a carotenoid biosynthesis inhibitor (NF), are also shown. While wild-type cotyledons contained normal chloroplasts with stacked thylakoid membranes within the entire chloroplast, two representative chloroplasts of *wco* (Fig. 3C, D) had considerably reduced thylakoid membranes, and essentially no stacked membranes were observed. Furthermore, these mutant chloroplasts had an unusual electron-sparse area, as well as over-accumulation of electron-dense particles (plastoglobuli). These features of the *wco* chloroplasts were not shared with the photobleached chloroplasts (Fig. 3B), which had few observable structural features within the double envelope membranes. Interestingly, the chloroplasts in the *wco* mutant cotyledons appear to resemble those of the *albino 3* mutant of *Arabidopsis*, although *albino 3* exhibits a similar phenotype in both cotyledons and true leaves (Sundberg et al. 1997).

Sucrose is required for survival of wco at the seedling stage—To test whether *wco* mutants also exhibit sugar-dependent growth similar to other mutants which have defects in chloroplast function or photosynthetic activity (e.g., Allison et al. 1996), the survival rate of *wco* under different sucrose concentration was analyzed. As shown in Table 2, sugar is clearly required for the survival of seedlings and development of true leaves. When germinated on a medium without sucrose, all the mutant seedlings died after cotyledon expansion before developing true leaves. Supplementing the medium with sucrose rescued the mutant seedlings, which then developed green true leaves. Once these leaves had developed, most of the mutant seedlings (more than 95%) could mature, flower, and produce fertile seeds in soil without sucrose supplementation (data not shown). Therefore, *wco* has non-functional chloroplasts only in the cotyledons, with those in the true leaves being active enough for autotrophic growth and completing the life cycle. The result also suggests that photosynthesis in normal cotyledons of *Arabidopsis* seedlings is essential for supplying a carbon source for true leaf initiation

Table 2 Survival of the *wco* seedlings depends on sucrose

	Sucrose concentration		
	0%	1%	3%
WT	1.00	1.00	1.00
wco	0.00	0.13	0.49

The calculated survival rate was based on about 100 seedlings tested under different sucrose concentrations as shown. Seedlings which failed to develop two expanded cotyledons were counted as non-germinated and excluded from this survival rate. Germination percentage was >95% in both cases.

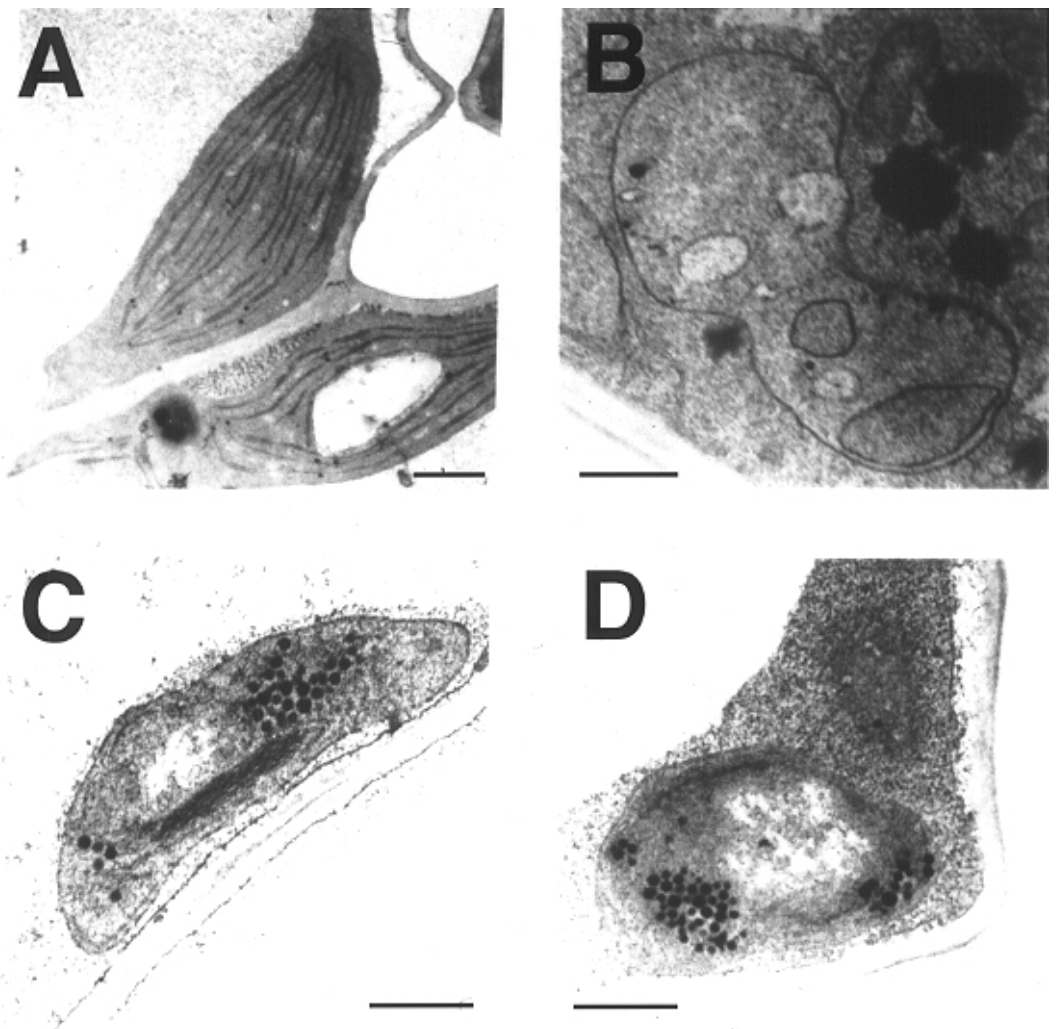

Fig. 3 The *wco* mutations result in severely underdeveloped chloroplasts in the cotyledons. Transmission electron microscopic images of chloroplasts in the cotyledons are shown. Bars indicate 0.5 μm in each panels. (A) wild-type. (B) wild-type treated with 100 nM NF. (C) and (D) *wco*.

and development.

The wco mutants exhibit a cotyledon-specific defect in chloroplast gene expression and 16S rRNA maturation— As a first step in revealing the molecular basis of the defect in the mutant chloroplasts, plastid gene expression of the 7-day-old mutant seedlings (only cotyledons and no true leaves) was analyzed by Northern hybridization (Fig. 4A). Compared with wild-type (lanes 1 and 3), *wco* (lanes 2 and 4) accumulated much less mRNA for *psbA* and *rbcL*, which encode the photosystem II reaction center and the large subunit of the ribulose 1,5-bisphosphate carboxylase, respectively. Quantification of the hybridization signals of the blots revealed that *wco* had as little as 3.4% and 3.8% of the wild-type level of *psbA* and *rbcL* transcripts, respectively. This result indicates that the expression of the chloroplast genes for photosynthetic components is severely inhibited in cotyledons of *wco*.

To examine the expression of non-photosynthetic genes encoded by the chloroplast genome, the accumulation profile of the chloroplast 16S rRNA was analyzed. As shown in Fig. 4A, while the overall level of the 16S rRNA was not significantly different between wild-type and the *wco* mutants, the relative abundance of the mature and unprocessed rRNA species changed significantly. In wild-type seedlings, most 16S rRNA is in the mature form with a lower molecular weight (Fig. 4A, 16S rRNA lower band). However, the *wco* seedlings had a much higher ratio of the pre-mature form to the mature one (Fig. 4A). This effect on 16S rRNA maturation is cotyledon-specific, since true leaves of *wco* mutants, which have functional chloroplasts, had almost no premature 16S rRNA, similar to that of wild-type (Fig. 4B). This result revealed that *WCO* is

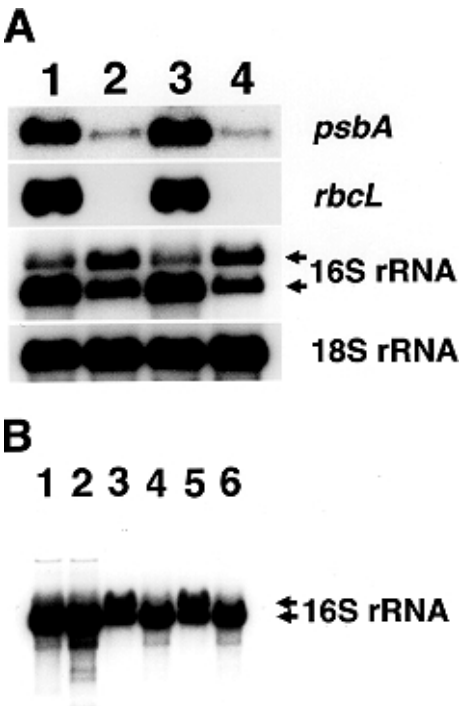

Fig. 4 Expression of plastid-encoded genes. (A) Plastid RNA accumulation at 7-day-old seedling stage. Lanes 1 and 3: two independent samples of wild-type; lane 2 and 4: mutant lines #9 and #19. The premature (upper band) and mature (low band) 16S rRNA are marked by arrows. The chloroplast genes *psbA* and *rbcL* encode the photosystem II reaction center protein and the large subunit of the ribulose 1,5-bisphosphate carboxylase, respectively. (B) Comparison of plastid 16S rRNA accumulation between seedlings (only cotyledons, 7-day-old, lanes 1, 3, and 5) or later stages which developed true leaves (3-week-old, lanes 2, 4, and 6. also see Fig. 2D). Lanes 1 and 2, wild-type. Lanes 3 and 4, *wco* (#9). Lanes 5 and 6, *wco* (#19). Equal loading was confirmed by the presence of the same amount of 18S cytosolic rRNA in each lane.

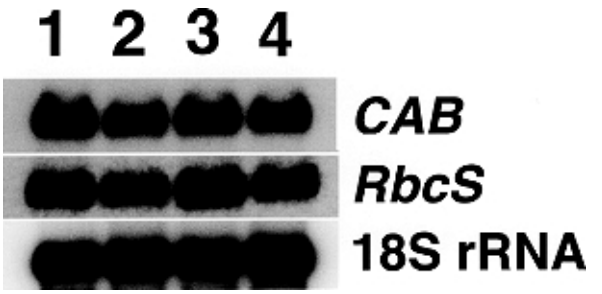

Fig. 5 Expression of nuclear-encoded genes for chloroplast proteins. Seven-day-old seedlings were harvested at noon and subjected to Northern analysis. Lanes 1 and 3, wild-type. Lane 2, the *wco* line #9. Lane 4, the *wco* line #19. *CAB* and *RbcS* are two nuclear genes encoding for the chlorophyll *a/b*-binding protein and the small subunit of the ribulose 1,5-bisphosphate carboxylase, respectively. Equal loading was confirmed by the presence of the same amount of 18S cytosolic rRNA in each lane.

indispensable for 16S rRNA maturation in a cotyledon-specific manner. This effect on 16S rRNA maturation has also been observed in one maize mutant, *hcf7*, which results in chloroplast translation deficiency due to inefficient incorporation of chloroplast mRNAs into polysomes leading to instability of chloroplast mRNAs (Barkan 1993). Therefore, the observed abnormal accumulation of premature 16S rRNA may suggest that the *wco* mutants have a primary lesion in 16S rRNA maturation or general RNA metabolism, which leads to a defect in translation and instability of chloroplast mRNAs and consequently dysfunctional chloroplasts.

The wco mutants do not inactivate the expression of nuclear-encoded genes for photosynthetic components—It has been well established that many nuclear genes for photosynthesis are suppressed by photo-oxidative destruction of chloroplasts caused by genetic mutations or feeding inhibitors for carotenoid biosynthesis (Oelmüller 1989). This suppression of the nuclear genes has been interpreted as a result of transduction of a so-called ''plastid signal'' from the dysfunctional chloroplasts to the nucleus (Mayfield and Taylor 1984, Susek and Chory 1992). To examine whether the dysfunctional chloroplasts in the mutant cotyledons affect the nuclear genes, the mRNA accumulation of *CAB* and *RbcS* in the light-grown *wco* mutant seedlings (without true leaves) was analyzed. As shown in Figure 5, the wild-type and the *wco* seedlings have the same level of *CAB* and *RbcS* mRNAs, indicating that the *wco* mutations do not affect expression of the nuclear genes for photosynthetic components. To further substantiate that there is no effect on the level of transcription of the nuclear photosynthesis-related genes, we examined the effect of the *wco* mutation on the *CAB1* promoter activity by introducing a CAB1::GUS reporter transgene into the *wco* mutant background. As shown in Figure 6, GUS staining revealed that CAB1::GUS expression in *wco* is comparable to the wild-type with regard to tissue specificity and the expression level in cotyledons (Fig. 6B, panel ''−NF''), further supporting the observations based on RNA blot analysis.

The wco mutations do not interfere with the effect of chloroplast oxidative destruction on silencing nuclear-encoded genes for photosynthetic components—The normal expression of the nuclear genes in *wco* cotyledons implies that the plastid signal for suppression of the nuclear gene expression is not activated in *wco*, or the signaling pathway itself is disrupted by the mutation of the *WCO* locus. In order to test which of the two possibilities is the case, we examined the effect of NF treatment on the CAB1::GUS expression in *wco* and wild-type seedlings. When wild-type seedlings were treated with NF, CAB1::GUS expression in wild-type cotyledons was strongly suppressed, demonstrat-

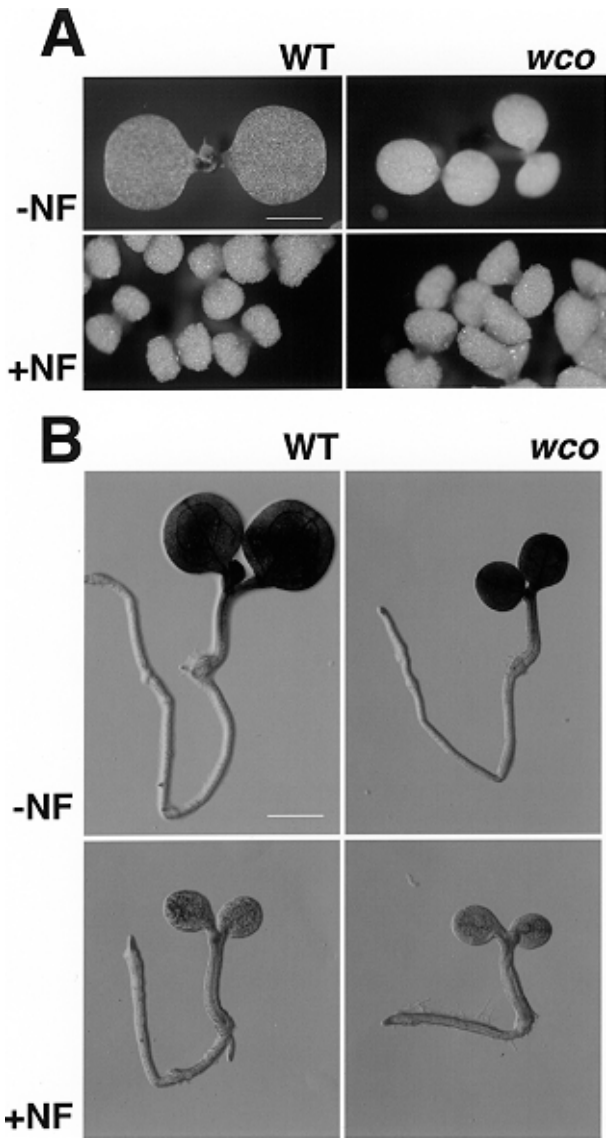

Fig. 6 Expression of CAB1::GUS transgene in the *wco* mutants. (A) A comparison of the 7-day-old seedlings of wild-type (WT) or *wco* mutants (homozygous CAB1::GUS transgene) treated with or without NF. (B) Histochemical staining of GUS activity (blue color). Note the similar tissue specificity and responsiveness to NF in both *wco* mutant and wild-type seedlings.

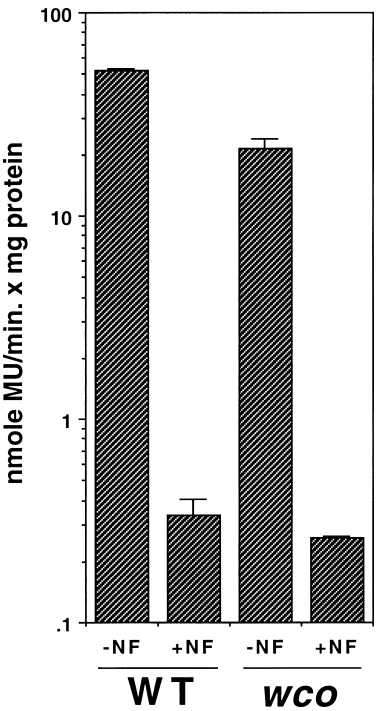

Fig. 7 The *wco* mutants are capable of responding to photo-oxidative damage caused by NF application. Expression of CAB1::GUS in the *wco* mutant and wild-type seedlings as shown in Fig. 6 was quantified and presented. The data from three independent experiments are shown with error bars indicating the standard deviation from the mean.

ing the activation of the ''plastid signal'' (Fig. 6B). The same treatment to *wco* also suppressed the CAB1::GUS expression in the cotyledons (Fig. 6B). Further quantitative analysis of GUS expression indicated that the NF treatment suppressed the CAB1::GUS expression to about 100-fold in both wild-type and *wco* (Fig. 7), further confirming the GUS activity staining pattern shown in Figure 6. Clearly, the *wco* mutants are fully capable of responding to the ''plastid signal'' and thus the corresponding signaling pathway should be intact. This result implies that the dysfunctional chloroplasts in the *wco* cotyledons are distinct from those induced by photo-oxidation and do not trigger the ''plastid signal'' to suppress nuclear genes for photosynthetic components.

Discussion

Differential requirement of WCO for chloroplast development between cotyledons and true leaves—The phenotype of the loss-of-function mutants demonstrates that *WCO* is required specifically for development of cotyledonous plastids. While it plays a role in true leaves, it is largely dispensable and the mutant plants can complete their life cycle photoautotrophically, indicating a differential requirement of the *WCO* gene for chloroplast development between cotyledons and true leaves.

There are several reports which indicate a difference between cotyledons and true leaves in respect to chloroplast biogenesis (Mansfield and Briarty 1996) and kinetic properties of light induction of nuclear genes (Thompson and White 1991). A cotyledon-specific *cis*-element for light activation of gene expression has also recently been identified from tobacco *PsaDb*, which is up-regulated during

chloroplast development ('internal LRE' in Yamamoto et al. 1995, 1997). These reports suggest that cotyledons and true leaves undergo distinct developmental programs for chloroplast biogenesis. This idea is not surprising because, unlike true leaves, cotyledons are specialized embryonic leaves where pre-existing plastids of intermediate form start differentiation in a synchronous way during seedling development (Mansfield and Briarty 1996). The identification of the *WCO* locus provides the genetic evidence for differential regulation of chloroplast development between these two leaf types.

Implication for the coordination of plastid development and nuclear gene expression—There are a number of reports demonstrating that defects in chloroplasts result in suppression of nuclear gene expression. For example, deletion of the chloroplast genome (Dunford and Walden 1991), suppression of chlorophyll biosynthesis (Kruse et al. 1995), reduction of carotenoids due to genetic mutation or the corresponding inhibitor application (Oelmüller 1989), mutation of chloroplast enzymes (Mandel et al. 1996, Sundberg et al. 1997), and a defect in translation at the chloroplasts (Hess et al. 1994), all result in partial or complete suppression of the *CAB* and/or *RbcS* gene expression. Based on these observations, a signal from the plastid to the nucleus the so-called ''plastid signal'' has been postulated. Genetic studies of *Arabidopsis* have identified several genetic loci which may be involved in this response (Susek et al. 1993), providing evidence for this hypothetical signal. The plastid signal is supposed to relay information about the oxidative stress (Oelmüller 1989) and/or activity state of chloroplasts (Kropat et al. 1997).

The *wco* mutants have defective chloroplasts in the cotyledons but normal expression levels of the nuclear genes *CAB* and *RbcS* (Fig. 4), uncoupling the nuclear gene expression and chloroplast development. Since the *wco* mutants are able to suppress the nuclear gene expression upon oxidative damage after NF application (Fig. 6, 7), the signaling pathway from photodamaged chloroplasts which shuts down nuclear gene expression should be intact in the mutants. These results draw a distinction between ''plastid signaling'' transmitting the developmental state of the chloroplast (e.g., Kropat et al. 1997) and the one mediating the photo-oxidative signal (e.g., Oelmüller 1989), because *wco* completely lacks the former response while is fully able to respond to the latter. One of the interpretations of this differential response of *wco* to the two hypothetical pathways is that mutation at the *WCO* locus might cause a specific block of the former signaling pathway. In this case, WCO is suggested to be a component of the signaling pathway. Alternatively, a separate pathway may not ever exist for the developmental state of chloroplasts. In this case, although *wco* has dysfunctional chloroplasts, they would not have sufficient photo-oxidation for the signal to be produced and transduced. As it is difficult to separate the above two hypothetical signaling pathways using wild-type or any other known mutants, availability of the *wco* mutants should provide a clear experimental system to address this question.

The wco mutants may have a primary lesion in plastid RNA metabolism—Our analysis of chloroplast RNA accumulation revealed that the *wco* mutants are defective in maturation of the chloroplast 16S rRNA (Fig. 4). This defect is very similar to that found in the maize *hcf7* mutant, which has pale leaves and accumulates premature 16S rRNA (Barkan 1993). Because this accumulation is specific for *hcf7* among several chloroplast mutants (Barkan 1993), this defect could be the primary result of the mutation at *HCF7* in maize and *WCO* in *Arabidopsis*. The pre-16S rRNA of maize *hcf7* is inefficiently incorporated into the chloroplast polysomes, displaying disfunctionality of the ribosomes containing the pre-16S rRNA (Barkan 1993). This defect may cause the translational inefficiency of many chloroplast mRNAs. In comparison with the maize *hcf7*, observed phenotypic changes of *Arabidopsis wco*, including dysfunctionality of chloroplasts and poor expression of the chloroplast genes, could be the result of the irregular accumulation of pre-16S rRNA. It is possible that *wco* may be the *Arabidopsis* counterpart of maize *hcf7*, albeit in a cotyledon-specific manner. It would be interesting to reveal the molecular identity of the *WCO* locus and its biochemical function in chloroplast development.

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