Pigment Deficiency in Nightshade/Tobacco Cybrids Is Caused by the Failure to Edit the Plastid ATPase α-Subunit mRNA

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The subgenomes of the plant cell, the nuclear genome, the plastome, and the chondriome are known to interact through various types of coevolving macromolecules. The combination of the organellar genome from one species with the nuclear genome of another species often leads to plants with deleterious phenotypes, demonstrating that plant subgenomes coevolve. The molecular mechanisms behind this nuclear–organellar incompatibility have been elusive, even though the phenomenon is widespread and has been known for >70 years. Here, we show by direct and reverse genetic approaches that the albino phenotype of a flowering plant with the nuclear genome of Atropa belladonna (deadly nightshade) and the plastome of Nicotiana tabacum (tobacco) develops as a result of a defect in RNA editing of a tobacco-specific editing site in the plastid ATPase α-subunit transcript. A plastome-wide analysis of RNA editing in these cytoplasmic hybrids and in plants with a tobacco nucleus and nightshade chloroplasts revealed additional defects in the editing of species-specific editing sites, suggesting that differences in RNA editing patterns in general contribute to the pigment deficiencies observed in interspecific nuclear–plastidial incompatibilities.

INTRODUCTION

The most fundamental step in the evolution of the eukaryotic cell was the endosymbiotic acquisition of organelles. The association of formerly free-living prokaryotes with the host cell was followed by a dramatic reorganization of the genomes of both the host and the symbionts. Among other processes, a substantial part of the genetic information of the prokaryotic endosymbionts was transferred into the nuclear genome (Dyall et al., 2004). Many products of these transferred genes have to be reimported into the organelles, and once there, they interact with organellar gene products. This type of interaction requires tight spatiotemporal regulation and leads to coevolution of the interacting compartments (Blier et al., 2001; Herrmann et al., 2003). To date, it has not been clear which interactions are evolutionarily conserved and which diversify more rapidly. Are there interactions that are specific to a certain taxon, or are most, if not all, interactions common among most species? A way to study these questions is to determine what happens when organelles are exchanged between species. It is interesting that interspecific organelle exchange often leads to malfunctions in cellular development and differentiation, a phenomenon referred to as nuclear–organellar incompatibility.

The initial characterization of nuclear–organellar incompatibility dates back to 1929, when Renner described aberrant pigment phenotypes of interspecies hybrids in the higher plant genus Oenothera (Renner, 1929, 1934; Stubbe, 1989). Similar studies throughout the plant kingdom showed that in many genera of angiosperms, interspecies hybrids and alloplasmic lines (cybrids) generated either by sexual crosses or by the fusion of somatic cells exhibit defective chloroplast proteins (Babychuk et al., 1995), chlorophyll deficiencies, and defects in chloroplast development (Hagemann, 1964; Kirk and Tilney-Bassett, 1987; Zubko et al., 2001; Levin, 2002). Nuclear–organellar incompatibility is also well documented in metazoans. In crustaceans, subpopulations of copepods exhibit differences in mitochondrial genes that cause a pronounced decline in cytochrome oxidase activity in interpopulation hybrids (Edmands and Burton, 1999). Similarly, cybrid cells containing human nuclei and mitochondria from different primate species vary in fitness (Kenyon and Moraes, 1997). The loss of normal organelle development in these models has been interpreted as a failure of the chondriome to function in a foreign genetic background. In molecular terms, the exchange of organelles between species can disrupt interactions between coadapted macromolecules, which can lead to cellular malfunction.

It has been estimated that up to 5000 unique nuclear genes encode proteins that are targeted to plastids (Martin and Herrmann, 1998; Peltier et al., 2000). Many nucleus-encoded plastid proteins...
participate in stable or transient interactions with plastid-encoded proteins or nucleic acids (Barkan and Goldschmidt-Clermont, 2000; Nickelsen, 2003). Intertaxonomic variability between such partners could trigger nuclear–organellar incompatibility. To locate differences in plastid genomes that potentially could be responsible for nuclear–organellar incompatibility, a genomic approach is inevitable. Comparative genomic analyses of plastomes can be used to identify all species-specific polymorphisms between different species that are able to produce natural or artificial cytoplasmic hybrids. Subsequently, the functional role of the identified polymorphisms in nuclear–organellar incompatibility can be tested by reverse genetics of the plastome, which of course requires a plant species amenable to plastid transformation. In addition, a forward genetic approach can be applied to isolate revertants from incompatibility, which requires easily distinguishable wild-type and nuclear–organellar incompatibility phenotypes.

Model organisms that meet these requirements and allow the study of the molecular mechanisms of nuclear–organellar incompatibility in higher plants are the cytoplasmic hybrids (cybrids) of *Atropa belladonna* (deadly nightshade) and *Nicotiana tabacum* (tobacco). Tobacco and nightshade are sexually incompatible species of the family Solanaceae but readily produce hybrids and cybrids in somatic cell hybridization experiments. Ab(Nt) cybrids with a nuclear genome Ab from nightshade and a plastome (Nt) from tobacco display an albino phenotype and are viable only when grown under heterotrophic conditions in vitro (Kushnir et al., 1991) (Figure 1A, Table 1). Plastids in mesophyll cells of Ab(Nt) plants are smaller than those in the wild type, have no normal thylakoid membrane system, and contain instead large vesicular structures (Herrmann et al., 2003). The easily scorable phenotype facilitates both direct and reverse genetic analyses. More importantly, targeted genetic manipulation of the plastome of one of the parental species, tobacco, is straightforward (Svab and Maliga, 1993). This, in combination with the somatic cell genetics available for both plant species, allows testing of the functional significance of species-specific polymorphisms for nuclear–organellar incompatibility. We sequenced the entire plastid chromosome from nightshade and compared it with the corresponding molecule from tobacco (Schmitz-Linneweber et al., 2002). The two chromosomes turned out to be 96% identical but were distinguished by >100 insertion/deletions in intergenic regions and several insertion/deletions and numerous point mutations in the coding regions of genes. Other polymorphisms affected nucleotide positions that are subject to RNA editing in corresponding transcripts (Schmitz-Linneweber et al., 2002). The wide variety of differences discovered suggested initially that multiple defects should

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**Figure 1.** Generation of Nightshade/Tobacco Somatic Hybrids and Mutants.  

(A) Scheme of the somatic cell hybridization experiments. Differential selection for nuclear and plastid genomes after the initial polyethylene glycol-induced formation of a heterokaryocyte leads to either the reciprocal cybrids Nt(AB) and AB(Nt) or the symmetric nuclear hybrids AB(Nt). Nuclei are represented by circles, and plastids are represented by ovals. Nuclear and plastid genomes contained within nuclei and plastids are coded Nt for tobacco and Ab for nightshade; plastid genome composition is indicated in parentheses.  

(B) Generation of Ab(Nt™) suppressor lines by means of the mutagenic agent NMU. The set of consecutive arrows denote several somatic cell fusion experiments detailed in Table 1.  

(C) Generation of an asymmetric radiation hybrid Ab(Nt™) carrying a limited amount of the tobacco nuclear genome. Before somatic hybridization, the tobacco nuclear genome was fragmented using γ-ray treatment.
underlie nuclear–organellar incompatibility. Here, we show that the albino phenotype in Ab(Nt) plants is caused primarily by the failure to edit a particular tobacco-specific plastid RNA editing site in the ATPase a-subunit (atpA) transcript. Further editing defects of species-specific sites were found in cybrids and may contribute to nuclear–organellar incompatibility to a lesser extent. These results emphasize an important role for the organellar RNA editing machinery in the evolutionary coadaptation of plastids and nuclei in higher plants.

### RESULTS

Cytoplasmically Inherited Mutations Suppress the Pigment Deficiency of Ab(Nt) Cybrids

To identify genes that underlie the nuclear–organellar incompatibility in Ab(Nt) cytoplasmic hybrids, we implemented a genetic approach to screen for plastid suppressor mutations of albinism.Suppressor mutations were detected by their ability to restore

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**Table 1. Genotypes of Parental Lines and Plants Used/Generated in This Study**

<table>
<thead>
<tr>
<th>GC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>ID&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Pigment Phenotype</th>
<th>Nuclear Genome</th>
<th>Plastome</th>
<th>Origin&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab(Ref)</td>
<td>Ab5</td>
<td>Green</td>
<td>nptII</td>
<td>–</td>
<td>Transgenic line, used as wild-type nightshade</td>
<td>Kushnir et al. (1991)</td>
</tr>
<tr>
<td>Nt(Nt)</td>
<td>SR1</td>
<td>Green</td>
<td>–</td>
<td>m16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Induced mutant, used as wild-type tobacco</td>
<td>Maliga et al. (1975)</td>
</tr>
<tr>
<td>Nt(Nt&lt;sup&gt;talb&lt;/sup&gt;)</td>
<td>A15</td>
<td>Albino</td>
<td>bar</td>
<td>m16&lt;sup&gt;a&lt;/sup&gt;, x</td>
<td>Plastome recipient</td>
<td>Svab and Maliga (1986)</td>
</tr>
<tr>
<td>Nt(Nt)</td>
<td>BarD</td>
<td>Green</td>
<td>bar</td>
<td>m16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Transgenic line, donor of nuclear genome</td>
<td>This study</td>
</tr>
<tr>
<td>Nt(Nt)</td>
<td>DS1B</td>
<td>Green</td>
<td>–</td>
<td>–</td>
<td>Cybrid, plastome of wild-type nightshade</td>
<td>Kushnir et al. (1987)</td>
</tr>
<tr>
<td>Nt(Nt)</td>
<td>Abw3</td>
<td>Albino</td>
<td>nptII</td>
<td>m16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Cybrid, plastome of tobacco cv Gatersleben</td>
<td>Kushnir et al. (1991)</td>
</tr>
<tr>
<td>Ab(Nt)</td>
<td>Abw3</td>
<td>Albino</td>
<td>–</td>
<td>–</td>
<td>Cybrid, plastome of tobacco cv Petit Havana</td>
<td>Kushnir et al. (1991)</td>
</tr>
<tr>
<td>Ab&lt;sup&gt;m&lt;/sup&gt;(Nt&lt;sup&gt;m&lt;/sup&gt;)</td>
<td>AG1/AG7</td>
<td>Green</td>
<td>nptII</td>
<td>m16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>→ NMU</td>
<td>AG1/7</td>
</tr>
<tr>
<td>Ab&lt;sup&gt;m&lt;/sup&gt;(Nt&lt;sup&gt;m&lt;/sup&gt;)</td>
<td>AG2</td>
<td>Green</td>
<td>–</td>
<td>–</td>
<td>→ NMU</td>
<td>AG2</td>
</tr>
<tr>
<td>Nt(Nt&lt;sup&gt;m&lt;/sup&gt;)</td>
<td>L3/7</td>
<td>Green</td>
<td>–</td>
<td>m16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>→ NMU</td>
<td>AG7 + A15</td>
</tr>
<tr>
<td>Ab(Nt&lt;sup&gt;m&lt;/sup&gt;)</td>
<td>L3</td>
<td>Green</td>
<td>nptII</td>
<td>m16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>→ NMU</td>
<td>L3/7 + Abw3</td>
</tr>
<tr>
<td>AbNt(Nt)</td>
<td>Ab27</td>
<td>Green</td>
<td>nptII</td>
<td>m16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>→ NMU</td>
<td>L3</td>
</tr>
<tr>
<td>Ab&lt;sup&gt;m&lt;/sup&gt;(Nt)</td>
<td>Bar103</td>
<td>Green</td>
<td>nptII, bar</td>
<td>m16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>→ NMU</td>
<td>Bar103</td>
</tr>
<tr>
<td>Nt(Nt:CCC)</td>
<td>KA7</td>
<td>Green</td>
<td>–</td>
<td>aadA</td>
<td>Made by plastid transformation, control line without point mutation in atpA</td>
<td>This study</td>
</tr>
<tr>
<td>Nt(Nt:TGG)</td>
<td>WAT</td>
<td>Albino</td>
<td>–</td>
<td>aadA</td>
<td>Made by plastid transformation, P264W mutation</td>
<td>This study</td>
</tr>
<tr>
<td>Nt(Nt:CTC)</td>
<td>CAT 6; 10</td>
<td>Green</td>
<td>–</td>
<td>aadA</td>
<td>Made by plastid transformation, P264L mutation in atpA</td>
<td>This study</td>
</tr>
<tr>
<td>Nt(Nt:AbC2)</td>
<td>C2A; 9</td>
<td>Green</td>
<td>–</td>
<td>aadA</td>
<td>Made by plastid transformation, line carries aadA and a nightshade rpoC2 gene</td>
<td>This study</td>
</tr>
<tr>
<td>Nt(Nt:NtC2)</td>
<td>C2N</td>
<td>Green</td>
<td>–</td>
<td>aadA</td>
<td>Recombinant line recovered during generation of C2A lines&lt;sup&gt;d&lt;/sup&gt;</td>
<td>This study</td>
</tr>
</tbody>
</table>

<sup>a</sup> GC, genome composition. Ab and Nt denote the nuclear genomes of nightshade and tobacco, respectively. (Ab) and (Nt) refer to their respective plastomes. (Ntalb) refers to a tobacco plastome that carries an unidentified mutation with albino phenotype. Superior m (m) denotes putative mutations induced in genomes. AbNt corresponds to a hybrid nuclear genome with complete complements of tobacco and nightshade nuclear genomes, as found in symmetric nuclear hybrids. Superior Nt (Nt) indicates the presence of a partial tobacco nuclear genome in asymmetric hybrids.

<sup>b</sup> Identification numbers of genetic lines as used in the laboratory.

<sup>c</sup> Origins of a given line and some of the relevant information are summarized. For the plant material generated in this study, schematic presentations of screenings followed to obtain the indicated genome compositions and identifications of actual parental lines used are shown.

<sup>d</sup> Nightshade-specific <i>rpoC2</i> sequences including the deletion were replaced with the respective tobacco sequences by homologous recombination during selection for C2A lines.
chloroplast differentiation and thus convert Ab(Nt) cybrids from white to green plants. Leaf explants of two independent Ab(Nt) lines were treated with the alkylating mutagen nitrosonium urea (NMU), which is known to have a strong mutagenic effect on chloroplast DNA (Hagemann, 1964), and then induced to regenerate shoots. Three independent green shoot cultures of lines with the genome composition Abm(Ntm) were thus established (m = mutated). The reversions from the albino to a green phenotype were stable. No albino shoots or sectors of albino tissue in leaves were ever observed over an extended period of micropropagation, implying that the Abm(Ntm) lines were homoplastomic. Thus, chemical mutagenesis with NMU allowed the isolation of genetic suppressors of pigment deficiency developed as a result of nuclear–organellar incompatibility in the Ab(Nt) cybrids. Compared with the normal vegetative development of Ab(Nt) and Nt(Ntm) plants, as well as the albino plants used for mutagenesis, all of the suppressor mutants were impaired in normal growth and leaf development, presumably because of a heavy mutational load of the nuclear genome.

To determine whether genetic suppressor mutation(s) are cytoplasmically inherited, plastids from the randomly chosen Abm(Ntm) line AG7 were first transferred into tobacco cells and then back to nightshade. The albino cytoplasmic tobacco mutant A15 (Table 1) served as a recipient of the AG7 plastids. In a protoplast fusion experiment, numerous green calli that regenerated tobacco-like shoots were selected. Tobacco plants of the presumed Nt(Ntm) genotype were healthy, fertile, and produced only green progeny after backcrossing with wild-type tobacco. This established that putative mutations in the plastome of the Abm(Ntm) line AG7 do not affect its ability to cooperate with the tobacco plastome in plastid chloroplast development. Next, plastids from one of the Nt(Ntm) lines (L3/7; Table 1) were combined with the nuclear genome of the original Ab(Nt) cybrid albino line Abw3. The resulting plants of the expected genotype Ab(Ntm) were green, photoautotrophic, and displayed normal vegetative development as well as nightshade morphology (Figure 1B). However, Ab(Ntm) plants were male sterile. Upon pollination with wild-type nightshade, normal-looking nightshade-like green progeny plants were obtained. Analysis of the Ab(Ntm) plants with regard to species-specific nuclear markers, such as tobacco-specific repetitive elements and isoenzyme types of peroxidases and amylases, did not reveal the presence of tobacco nuclear genes (see Supplemental Figure 1 online). Based on the results of plastid transfer experiments and because the cytoplasmic genomes of both species are maternally inherited in sexual crosses, we concluded that suppressor mutations are borne by the cytoplasmic genome, most likely the plastome, and that these mutations enable cooperation between the tobacco plastome and the nightshade nuclear genome to develop functional chloroplasts.

**Suppression of Albinism Is Associated with a C-to-T Point Mutation at Plastid Codon atpA-264**

To identify the suppressor mutations, we determined partial nucleotide sequences of the mutated tobacco plastid chromosomes. We focused on previously identified polymorphic sites between the tobacco and nightshade plastid chromosomes, among which the most striking were differences in RNA editing sites (Schmitz-Linneweber et al., 2002). Analysis of the five tobacco-specific RNA editing sites showed that the plastomes of all three genetically suppressed Abm(Ntm) lines carried a C-to-T base substitution in the middle of the 264th CCC codon of the atpA gene, which codes for the a subunit of the plastid ATP synthase. For each line, 1.5 kb was sequenced adjacent to the tobacco-specific editing sites. Three further point mutations were found, each of them unique to a different line and none of them affecting coding, which corroborates the independent origin of the genetically altered plastid chromosomes. The cytosine base in question is edited in wild-type tobacco atpA mRNAs to uracil, thus converting a CCC-Pro codon to a CUC-Leu codon. In nightshade atpA, a Leu is already encoded at the DNA level by a CTC codon (Schmitz-Linneweber et al., 2002). Thus, the identified mutation did not alter the amino acid sequence of AtpA; it just eliminated the need for RNA editing.

To understand how the identified mutation may contribute to the suppression of the albino phenotype of Ab(Nt) plants, we next analyzed the editing of the atpA transcript. We found that Ab(Nt) plants fail to edit the atpA transcript (Table 2). As all previous evidence suggests that editing factors are encoded in the nucleus (Bock, 2000), these data indicate that the nightshade nuclear genome lacks the gene that codes for an appropriate editing factor for this site. Thus, codon 264 remains CCC in the atpA mRNA, suggesting that the nascent AtpA polypeptide in Ab(Nt) cybrids contains a L264F amino acid substitution. Consequently, the characterized suppressor mutations remove the atpA editing site, which should restore the Leu in the AtpA protein, thus compensating for nightshade’s lack of tobacco-specific nucleus-encoded editing factors. These data suggested that the Leu-264 residue in the AtpA polypeptide is indispensable for the proper development of chloroplasts, because all three independently isolated suppressor lines possessed an identical mutational change affecting this codon.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>mRNA</th>
<th>Tobacco-Specific Editing Sitesa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>atpA</td>
</tr>
<tr>
<td>Nt(Nt)</td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>Ab(Ab)</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>Ab(Nt)</td>
<td>Albino</td>
<td>+</td>
</tr>
<tr>
<td>Ab(Ntm)</td>
<td>Green</td>
<td>+</td>
</tr>
<tr>
<td>Ab(Nt:CTC)</td>
<td>Green</td>
<td>-</td>
</tr>
<tr>
<td>Ab(Nt:CCC)</td>
<td>Albino</td>
<td>-</td>
</tr>
<tr>
<td>Nt(Lrp-0)</td>
<td>Albino</td>
<td>+/−</td>
</tr>
</tbody>
</table>

* a, Site fully edited; −, site not edited; +/−, site partially edited; T, thymidine in DNA. Excerpts of corresponding cDNA sequence chromatograms are shown in Supplemental Figure 2A online.

b C is the edited nucleotide in tobacco.

c Encoded amino acid residue after editing.
**AtpA Leu-264 Is Essential for Plastid Development**

To verify the functional role of AtpA Leu-264, we applied homologous recombination-mediated gene replacement to mutate the codon for this amino acid residue. An initial attempt to mimic the editing defect in Ab(Nt) plants failed. The \textit{cis} sequence proximal to the editing site, which are known to be important for the binding of editing factors (Bock et al., 1996; Chaudhuri and Maliga, 1996; Miyamoto et al., 2002), were mutated, but the recovered mutant plants, designated mA lines, still partially edited the atpA message (see Supplemental Figure 2B online) and were indistinguishable from control tobacco plants. Next, we introduced a P264W codon substitution into the atpA gene. To generate tobacco plants with the genomic composition Nt(Nt:TGG), where TGG denotes the replacement of the CCC codon for Pro with the TGG codon for Trp at position 264, we used biologic plastid transformation (Figure 2A). The presence and homoplasy of the altered plastid chromosomes was verified by sequence and restriction analysis (Figure 2B). Lines of transgenic tobacco plants, designated WAT, which were homoplasmic for the codon substitution, were albino and could only be propagated heterotrophically (Figure 2E). By contrast, control plants carrying the marker gene \textit{aadA} used to select transplastomic plants but without the point mutation, with genome composition Nt(Nt:CCC), were indistinguishable from wild-type plants (Figure 2E). This shows that amino acid residue 264 of the AtpA polypeptide is critical for chloroplast development and hence for plant survival. Consequently, the CCC-to-CUC codon exchange generated by RNA editing at this position is likely to affect protein function as well, which is in agreement with the significance of editing for the function of several other plastid-encoded proteins (Bock et al., 1994; Hirose and Sugiura, 1997; Zito et al., 1997; Sasaki et al., 2001).

**Restoration of Greening by Genetic Introgression of Tobacco Nucleus-Encoded Editing Factors**

If nonediting of the \textit{atpA}-264 site is responsible for the albino phenotype of Ab(Nt) plants, then genetic complementation of the mutant phenotype should be achieved by introducing tobacco nuclear genes that code for the \textit{atpA} editing factors. We used somatic cell hybridization to generate symmetric and asymmetric nuclear hybrids to test this possibility. Symmetric nuclear hybrids, AbNt(Nt), which possess nuclear genomes of both species and the plastome of tobacco, were phenotypically intermediate compared with their parental species (Figure 1A). These plants were photoautotrophic, partially fertile, and their \textit{atpA} transcript was fully edited, as expected (see Supplemental Figures 2 and 3 online).

Next, a panel of asymmetric nuclear hybrids with a full chromosome set of nightshade and a partial genomic complement of tobacco was generated. In this somatic hybridization experiment, cells of herbicide (BASTA)-resistant tobacco were irradiated with a lethal dose (500 GY) of \textit{γ}-rays and fused with protoplasts of the albino cybrid Ab(Nt) (Table 1). BASTA-resistant green colonies were selected, and regenerating shoots were screened for the absence of tobacco-specific morphological traits. The morphology of symmetric tobacco/nightshade hybrids served as a reference. Specifically, the counterselected traits included the absence of (1) abundant trichome development (i.e., hirsuteness, a typical tobacco trait); (2) characteristic tobacco leaf shape; and (3) typical morphology of the tobacco root system. The selected line Bar103 with the expected genotype Ab\textit{Nt}(Nt) displayed nightshade shoot and leaf morphology (Figure 1C, Table 1) and developed greenish adventitious roots, a nightshade trait. Pollination of flowers of Bar103 plants with wild-type nightshade pollen yielded only progeny that were purely albino. Most likely, the tobacco nucleus genome fragments were not transmitted to the F1 progeny plants as a result of their loss during meiosis. The albino phenotype of F1 progeny plants further suggests that the tobacco plastome in Bar103 is wild type, as it is still incompatible with the nightshade nucleus genome. Ab\textit{Nt}(Nt) plants displayed a fully edited \textit{atpA} codon 264 (Table 2). This established that the introduction of tobacco nuclear DNA can complement the albinism of Ab(Nt) plants and that this complementation correlates with the plant’s ability to edit the \textit{atpA} transcript.

**Removal of the \textit{atpA}-264 Editing Site Restores Greening**

We could not exclude the possibility that other point mutations in the plastid chromosome of Ab(Nt\textsuperscript{m}) plants play a role in the restoration of plastid differentiation, in addition to the C-to-T mutation at the \textit{atpA} editing site (Figure 1B). Therefore, we set out to test whether a P264L codon substitution in tobacco \textit{atpA} alone would suffice to restore the compatibility between the nightshade nucleus genome and the tobacco plastome. Several attempts at direct plastome transformation of the albino Ab(Nt) cybrid were not successful. In an alternative approach, the tobacco plastid chromosome was modified within tobacco and the compatibility of the engineered plastome with the nightshade nucleus was assessed in a second step by interspecific plastid transfer experiments. We constructed transplastomic tobacco CAT lines, with genomic composition Nt(Nt:CTC), with a single C-to-T point mutation leading to a P264L codon substitution at the DNA level (Figure 2A). Line KA7, which had the genomic composition Nt(Nt:CCC), served as a control; it carried only the \textit{aadA} cassette but no point mutation. The transplastomic plants were used as donors of plastids in protoplast fusion experiments with nightshade as the plastid recipient.

In the control protoplast fusion experiment using a kanamycin-resistant nightshade strain and line KA7, 16 Ab(Nt:CCC) albino lines were recovered that were indistinguishable from Ab(Nt) plants generated previously (Table 3, Figure 2E). By contrast, four independent fusion experiments with protoplasts of CAT\textit{b} and CAT10 tobacco lines led to the generation of 32 lines of cybrids that were all green and essentially indistinguishable from Ab(Nt\textsuperscript{m}) plants (Table 3, Figure 2E). They grew on soil with growth rates similar to those of nightshade wild-type plants, demonstrating photoautotrophy. Sequence and restriction analyses of the \textit{atpA} gene in newly generated cybrids confirmed the presence and absence of the point mutation in Ab(Nt:CTC) and Ab(Nt:CCC) plants, respectively. When we used tobacco line CAT8 as a plastid donor, a line that was heteroplasmic for the mutation (Figure 2C), both green and albino nightshade cybrids were recovered. Sequence and restriction analyses showed that
Figure 2. Reverse Genetic Analysis of Codon 264 of the atpA Gene.
only the green cybrids contained the C-to-T point mutation (Figure 2D; see also Supplemental Figure 4 online).

Whereas cybrid plants did not differ significantly from wild-type nightshade in their vegetative growth under our standard greenhouse conditions, independent lines varied in fertility. Of 12 lines tested, only 2 were self-fertile. Other lines were female fertile (i.e., developed seeds after pollination with pollen of wild-type nightshade) but male sterile. The male sterility of cybrids varied. Plants of some lines had normal flowers with developed anthers that, however, did not contain viable pollen upon dehiscence. In other lines, petaloid anthers were common. Such abnormalities in flower development are the basis for so-called cytoplasmic male sterility. They are common among cytoplasmic hybrids of plants and are thought to be caused by disturbed nuclear–mitochondrial interactions (Nivison and Hanson, 1989; Yesodi et al., 1997). However, the recovery of two fully fertile, photoautotrophic Ab(Nt:CTC) cybrid lines demonstrates that nuclear–plastidial incompatibility between the nightshade nuclear genome and the tobacco plastid genome manifested as pigment deficiency can be directly overcome by a single point mutation.

Albinism in Ab(Nt) Cybrids Is Not Overcome by the Introduction of a Nightshade-Type Polymorphism into the Tobacco rpoC2 Gene

The ancient eubacteria-like plastid-encoded RNA polymerase (PEP), one of the RNA polymerases active in the chloroplast, is composed of both nucleus- and plastid-encoded subunits (Suzuki et al., 2004). A comparative genomic analysis of tobacco and nightshade plastomes has shown that the rpoC2 gene, which encodes the PEP β’’ subunit, has a three-codon deletion in nightshade compared with tobacco (Schmitz-Linneweber et al., 2002). Hence, it is conceivable that in Ab(Nt) cybrids, a tobacco β’’ subunit fails to interact properly with nightshade nucleus-encoded subunits. Therefore, it was possible that compromised PEP activity in Ab(Nt) plants contributes to albinism, because tobacco loss-of-function mutants of PEP are albino (Allison et al., 1996; De Santis-Maciossek et al., 1999). This proposal was supported by data from in vitro experiments in which a partial recovery of chloroplast differentiation was obtained after microprojectile-mediated delivery of the nightshade rpoC2 gene into leaf cells of Ab(Nt) plants (Herrmann et al., 2003). However, in spite of numerous attempts, stable, homoplastomic lines of green revertants were not established in these experiments.

To test a possible role of the interspecies rpoC2 polymorphism for nuclear–organellar incompatibility in planta, we implemented the same experimental strategy that was used to assess the role of the tobacco atpA-264 editing site. First, we generated two types of transplastomic tobacco plants. Plants with the genome composition Nt(Nt:AbC2), lines C2A-2 and C2A-9, had a tobacco plastome in which the rpoC2 gene carried the same three-codon deletion as the rpoC2 gene of nightshade and was linked with the aadA selection cassette inserted 30 bp downstream of the rpoC2 stop codon. Plants with the genome composition Nt(Nt:NtC2), line C2N, possessed a control tobacco plastome that carried the selectable marker aadA linked to the rpoC2 gene containing the three tobacco-specific codons (Figure 3A). Both types of transplastomic tobacco plants grew photoautotrophically in soil but were slightly paler green than the wild-type plants (see Supplemental Figure 5 online). This suggested that the aadA cassette at this chromosomal position has some negative effect on chloroplast development, for example, by compromising the proper termination, stability, or translation of the rpoC2 transcript. Notwithstanding this slight pigment deficiency, we used these transplastomic plants as donors of plastids in protoplast fusion experiments with nightshade as the plastid recipient. All recovered lines of cybrids were albino regardless of genome composition, which was either Ab(Nt:AbC2) or Ab(Nt:NtC2), as verified by sequence analysis (Figure 3B, Table 3). These results suggested that the interspecific divergence in rpoC2 is not responsible for the albinism phenotype of Ab(Nt) cybrids.

Additional RNA Editing Defects of Species-Specific Sites in Ab(Nt) and Nt(Ab) Cybrids

Although the presented results unambiguously show that the editing defect in the atpA mRNA of Ab(Nt) cybrids can by itself explain albinism, this does not exclude the possibility that additional defects, such as further RNA editing defects, make a minor contribution to nuclear–organellar incompatibility in these plants. Therefore, we next analyzed the RNA editing of all known tobacco editing sites in Ab(Nt) plants. Among the five tobacco-specific editing sites (Schmitz-Linneweber et al., 2002),...
in addition to atpA-264, sites ndhD-200 and ndhD-225 were not edited, whereas sites psbE-72 and rps14-50 were completely and partially edited, respectively (Table 2). Editing of all of the other 29 sites shared between the two species was not affected (see Supplemental Figure 7 online). The editing failures found are not a pleiotropic consequence of albinism, because the five tobacco-specific sites are edited in albino tobacco mutants disrupted in the plastid rpoA or rpoB genes (Table 2) (De Santis-Maciossek et al., 1999). In contrast with the atpA site, sites ndhD-200 and ndhD-225 are edited only partially in nonphotosynthetic tissue (Chateigner-Boutin and Hanson, 2003). However, a complete abolishment of editing at these sites as observed in the Ab(Nt) cybrid has not been reported.

It is interesting that the asymmetric hybrid AbN(Nt) showed differential rescue of editing of the sites affected in Ab(Nt). In addition to atpA-264, editing at site rps14-50 was fully restored, whereas editing at site ndhD-225 was restored only partially, and no restoration occurred at site ndhD-200 (Table 2). Whether this means that several independent editing factors have been introduced in AbNt(Nt) plants, or alternatively, that one factor serves several sites, remains to be determined. In contrast with AbNt(Nt) plants, no alteration in editing of additional sites took place in the rescued Ab(Nt:CTC) cybrids. Here, editing of rps14 was still partial and no editing of the two ndhD sites was observed (Table 2). This shows that editing of these additional sites is not dependent on the status of the atpA site and that the editing defect of these sites does not contribute significantly to albinism in the Ab(Nt) plants.

We also investigated editing of the three nightshade-specific sites ndhA-189, ndhD-293, and rpoB-809 in the reciprocal Nt(Alb) cybrid (Figure 1A) and found that site rpoB-809 is not processed.
Abnormal RNA Editing Characterizes Cytoplasmic Hybrids in Solanaceae

In this study, direct and reverse genetic analyses were implemented to understand which of the many interspecies polymorphisms between the tobacco and nightshade plastomes are responsible for the albino phenotype of the cytoplasmic hybrid plants Ab(Nt). The results demonstrate that the inability of the nightshade nuclear genome to support effective editing of the tobacco atpA transcript in plastids is the primary cause of the observed pigment deficiency.

Although the editing defect of the atpA-264 site is clearly the most important component of incompatibility in the Ab(Nt) cybrids, other editing defects encountered in cybrids analyzed here might contribute to incompatibility on a smaller scale. AbB(Nt), Ab2(Nt), and Ab1(Nt:CTC) plants all exhibited different combinations of editing defects at the tobacco-specific sites rsps14-50, ndhD-200, and ndhD-225. Although all of these plants grew photoautotrophically, we cannot exclude the possibility that their RNA editing defects result in more subtle phenotypic alterations, such as a reduction in photosynthetic efficacy under certain stress conditions. The ndhD gene codes for a subunit of the Ndh complex, which participates in the reduction of plastoquinones and cyclic electron flow around photosystem I. The Ndh complex supplies extra ATP for photosynthesis, particularly under environmental stress conditions, but it is not essential for basic photosynthetic competence, because loss-of-function mutants are viable and their growth does not differ significantly from that of wild-type plants (Burrows et al., 1998; Kofer et al., 1998; Shikanai et al., 1998; Horvath et al., 2000; Kotera et al., 2005). The plastid rsps14 gene encodes a subunit of the ribosome. A knockout mutation of rsps14 is lethal (Ahlert et al., 2003), and thus even the partial rsps14-50 editing defect might impinge on normal plastid function in some subtle manner. Similarly, the rpsB-809 defect found in the Nt(AB) cybrid could play a role in the incompatibility syndrome observed in these plants (Babiychuk et al., 1995; Peter et al., 1999). This is supported by the finding that null mutants of rpsB, which encodes the β subunit of the plastid RNA polymerase, are albino plants (Allison et al., 1996; De Santis-Maciossek et al., 1999), although the amino acid Leu-809 is in a poorly conserved region of the protein and its functional importance remains to be validated (Corneille et al., 2000).

Together, the results of our plastome-wide analysis of RNA editing in cybrids are consistent with a complex polygenic nature of nuclear–organellar incompatibility in plants (Yao and Cohen, 2000; Zubko et al., 2001). It will be important to understand the functional role of rsps14-50, ndhD-200, ndhD-225, and rpsB-809 editing sites for plant growth and development. An extreme nuclear–organellar incompatibility reaction like the pigment deficiency of Ab(Nt) plants is rare in sexual hybrids. If RNA editing defects decrease the fitness of hybrids in natural environments, they most likely do so in a more subtle way, for example, by slightly decreasing the overall efficiency of photosynthesis.

Are editing defects expected to be a general feature of nuclear–organellar incompatibility? The loss of editing in a nightshade nuclear background can be explained by the absence of genes coding for species-specific editing factor. As all available evidence suggests a proteinaceous nature of editing factors (Miyamoto et al., 2002; Kotera et al., 2005), it follows that nuclear–organellar incompatibility in Ab(Nt) cybrids is the result of impaired protein–RNA interactions. Several studies suggest that these interactions may be evolutionarily labile. Editing sites evolve with extreme rapidity (Shields and Wolfe, 1997; Sasaki et al., 2003). In addition, the responsible nucleus-encoded RNA editing factors are evolutionarily labile as well (Bock et al., 1994; Bock and Koop, 1997; Reed and Hanson, 1997). This rapid evolution leads to interspecific divergence of editing sites and factors; thus, an exchange of organelles between species should be followed by editing defects. This prediction can be further tested in interspecific hybrids exhibiting nuclear–organellar incompatibility, for example, in the genera Oenothera and Epilobium (Renner, 1934; Michaelis, 1954; Kirk and Tilney-Bassett, 1987).
Can Plants Develop Normally Without Plastid RNA Editing Sites?

Functionally, the plastid RNA editing machinery appears to be indispensable for plants, because it is required to generate start codons and to avoid the production of nonfunctioning or malfunctioning proteins. This is supported by the finding that RNA editing restores codons for conserved amino acid residues (Bock et al., 1994; Hirose and Sugiiura, 1997; Zito et al., 1997; Sasaki et al., 2001; Kotera et al., 2005). Physiologically, there is still no satisfactory explanation for the existence of RNA editing in plant organelles. In contrast with metazoans (Turelli and Trono, 2005), there is no known example in which alternative editing in plants conditions physiological processes. The Nt(Nt:CTC) transgenic tobacco lines, in which the AtpA Leu-264 residue is encoded in DNA directly, demonstrate that the removal of the atpA-264 editing site does not entail any visible disadvantage for these plants when grown under standard laboratory conditions. The normal phenotype of Nt(Nt:CTC) plants suggests that the editing event per se is dispensable, because a C-to-T point mutation at the DNA level serves the same purpose (i.e., restoration of the appropriate conserved codon at this position). It will be interesting to determine whether this is true for all plastid editing sites and thus to determine whether plastid RNA editing is not a beneficial but rather a selfish process (Covello and Gray, 1993).

Male Sterility in Cytoplasmic Hybrids

The majority of Ab(Nt:CTC) cytoplasmic hybrids generated in this study were male sterile. Nuclear–organellar incompatibilities in higher plants are known to include cytoplasmic male sterility, which is an agriculturally important trait associated with aberrant mitochondria (Melchers et al., 1992; Conley and Hanson, 1995; Kofer et al., 1999). Cytoplasmic male sterility attributable to nuclear–mitochondrial incompatibility is thought to occur as a result of rearrangements of the mitochondrial genome presumably caused by illegitimate recombination (Belliard et al., 1979; Hanson and Bentollia, 2004). In addition, a function of RNA editing in cytoplasmic male sterility development has been proposed in rice (Oryza sativa), sorghum (Sorghum bicolor), and maize (Zea mays) (Iwabuchi et al., 1993; Howad et al., 1999; Gallagher et al., 2002). Compared with plastids, plant mitochondria edit their transcriptome to a greater extent. For example, 441 editing sites are present in the Arabidopsis thaliana mitochondriome (Giege and Brennicke, 1999). However, with the exception of the quantitative difference in editing sites, the editing systems in plastids and mitochondria share many features and are believed to be related (Maier et al., 1996). Thus, our results with plastid editing in nuclear–organellar incompatibility suggest that editing defects may occur in mitochondria of cytoplasmic hybrids as well. It will be interesting to determine whether this assumption is true and, if so, how such editing defects affect plant development.

Albinism in Ab(Nt) Plants

Previous studies have suggested that compromised protein–protein interactions act as triggers for nuclear–organellar incompatibility. In metazoans, nuclear–mitochondrial incompatibility has been explained by disrupted interactions of organellar and nucleus-encoded subunits of respiratory membrane complexes (Blier et al., 2001; Rawson and Burton, 2002). In plants, it has been speculated that the failure to properly assemble plastid proteases (Babiychuk et al., 1995) or photosynthetic complexes (Herrmann and Possingham, 1980; Zubko et al., 2001) marks an initial molecular event in the development of nuclear–plastidial incompatibility. In the case of Ab(Nt) cybrids, we previously suspected that the failure to assemble a functional PEP is responsible for the albino phenotype (Herrmann et al., 2003). Our reverse genetic analysis of the rpoC2 species-specific polymorphism did not confirm this idea, although it does not exclude the possibility that PEP function may be abnormal in Ab(Nt) cybrids. We do not know how green sectors developed on leaves of Ab(Nt) plants subjected to microprojectile-mediated delivery of the nightshade rpoC2 gene (Herrmann et al., 2003). Another open question is how L264W and L264P amino acid substitutions in the AtpA polypeptide result in the development of albino phenotypes in cybrids or transplastomic plants. Pharmacological inhibition of the ATP synthase with tentoxin phenocopies the albino phenotype of Ab(Nt) or Nt(Nt:TGG) plants (Holland et al., 1997). This indicates that the ATPase defect can directly cause the block of plastid differentiation and further suggests that the AtpA Leu-264 residue could be required for ATP synthase operation rather than causing an assembly problem. The genetic lines developed in our study can be exploited to understand how the inhibition of ATP synthase leads to the abortion of plastid differentiation.

In conclusion, the combination of experimental approaches implemented in this study helped to unravel the molecular mechanism behind pigment deficiency as a manifestation of nuclear–organellar incompatibility in Ab(Nt) plants. In general, these techniques should be applicable to the study of molecular processes that initiate nuclear–cytoplasmic incompatibilities in various families of higher plants and will advance our understanding of the role that cytoplasmic genomes play in the life cycle and evolution of plants.

METHODS

Chemical Mutagenesis of Leaf Explants

Chemical mutagenesis of somatic cells was performed essentially as described previously (McCabe et al., 1989). Two independent experiments were performed with the albino cybrid lines Abw and Abw3, which possess the plastome of tobacco cv Gatersleben and cv Petit Havana, respectively (Kushnir et al., 1991). Approximately 400 leaf explants, measuring 0.5 × 0.5 cm, were incubated for 1 h in liquid MS medium (Sigma-Aldrich, Munich, Germany) supplemented with 5 mM NMU. They were then thoroughly washed with sterile water and cultured in Petri dishes containing agar-solidified MS medium supplemented with 30 g/L sucrose, 0.5 mg/L 6-benzylaminopurine (Sigma-Aldrich), and 0.1 mg/L α-naphthalene acetic acid (Sigma-Aldrich) to induce somatic organogenesis. Regenerating shoots were scored for the appearance of green leaf sectors that were excised to establish stable lines of putative revertants by the induction of de novo shoot organogenesis followed by micropropagation.
Generation of Somatic Hybrids

Genotypes of the plant material used are summarized in Table 1. Leaf protoplast isolation, fusion, and culture were performed as described previously (Kushnir et al., 1991). Selectable markers used in protoplast fusion experiments (Table 1) were the kanamycin resistance gene cassette (pNPTII), the resistance gene for the herbicide BASTA (bar), a mutated version of the tobacco 16S chloroplast RNA gene that confers resistance to streptomycin (rmT16S), an unknown tobacco albino mutation with maternal (cytoplasmic) inheritance (X), and the spectinomycin resistance gene cassette (Koop et al., 1996). To generate symmetric nuclear nightshade (Atropa belladonna) tobacco hybrids with the tobacco plastome, leaf protoplasts of the streptomycin-resistant tobacco line SR1 (Maliga et al., 1975) were fused with leaf protoplasts of the kanamycin-resistant nightshade line Ab5 (Kushnir et al., 1991). Calli double resistant to streptomycin (500 μg/mL) and kanamycin (50 μg/mL) were selected and induced to regenerate shoots.

The glufosinate ammonium–resistant transgenic SR1 tobacco line BarD was generated using the published leaf disc transformation procedure using Agrobacterium tumefaciens C1Rfr (pGV2260; pGFP129). To generate asymmetric nuclear hybrids (Table 1), leaf protoplasts of BarD plants were irradiated with γ-rays from a Co60 source. The ionizing radiation dose used, 500 Gy, completely prevents cell division (i.e., is lethal). Irradiated BarD protoplasts were fused with nonirradiated leaf protoplasts of the albino nightshade Abw3 line. Such pretreatment usually results in the elimination of the donor nuclear genome after the first divisions of heterokaryocytes (Menczel et al., 1982). Glufosinate ammonium–resistant green calli were selected and induced to regenerate shoots.

To generate nightshade cybrids with plastids from transplastomic tobacco lines, we fused leaf protoplasts of the kanamycin-resistant nightshade line Ab5 with leaf protoplasts of tobacco lines as summarized in Table 3. Three-week-old colonies obtained after protoplast fusion were then cultured on media supplemented with kanamycin (100 μg/mL) and spectinomycin (1200 μg/mL). Nightshade-like shoots regenerated from independent colonies that developed under such selection were excised and micropropagated without selection. In the protoplast fusion Abw3–CAT6, only kanamycin was present during selection and green colonies were selected, because the parental nightshade cybrid line was albino.

Plants were grown aseptically with 8 h/16 h dark/light cycles at 0.5 to 1 W/m² (Osram L85 W/25 Universal White fluorescent lamps; Munich, Germany) and 25°C on synthetic MS medium that was supplemented with sucrose (30 g/L) and solidified with 0.6% agar. Species-specific polymorphic markers of nuclear genomes, such as isoenzymes of peroxi-

hexanucleotide primers using Superscript II (Invitrogen, Carlsbad, CA) and a TGG–ATG codon instead of the wild-type CCC–Pro codon at the atpA codon position 264. A mixture of 20 ng of each purified PCR product served as a template for further PCR with primers HatpAfor and HatpArev. The resulting DNA fragment was digested with BstTII and Mva1269I, purified by gel electrophoresis, and used to replace the identical sequence in pKA, resulting in the plasmid pWAT. Analogous experiments were performed to generate the vectors pCAT and pAmA, with the difference that the primers WATfor and WATrev for introducing point mutations were replaced by CATfor and CATrev or Mu-edaptAfor and Mu-edaptArev, respectively. The presence of the mutations and the absence of other mutations haphazardly introduced by PCR were verified by nucleotide sequence analysis. To generate a clone for the introduction of nightshade rpoC2 sequence into the tobacco plastid chromosome, a NcoI–BamHI fragment covering the sequence interval between nucleotide positions 15,055 and 18,710 of the nightshade plastid genome (GenBank accession number AJ316582) was subcloned into pBluescript SKII– (Stratagene). The aadA cassette was inserted into a unique EcoRI site downstream of rpoC2 at position 16,920, following the transcriptional direction of rpoC2. This vector was designated pC2A.

Plastid Transformation and Selection for Transplastomic Lines

Leaves of 14-d-old axenically grown tobacco seedlings were bombarded with plasmid DNA-coated gold particles using a bioisotonic device (PDS-1000/He system; Bio-Rad, Hercules, CA). Spectinomycin-resistant shoots were selected on RMOP medium containing 500 mg/L spectino-

mycin dihydrochloride (Svab and Maliga, 1993). Plastid transformants generated with pKA, pWAT, pAmA, and pCAT were identified by PCR using primer pairs P1–P2 and P3–P4, which also test integration polarity into the plastid chromosome. Mnr1 digestion of the amplification product derived with the primer pair EatpAfor–EatpArev was used to estimate the presence of the mutation as well as the transplastome: wild-type plastid chromosome ratios.

Plastid transformants generated with pC2A were identified by PCR using primer pairs rpoC2for and rpoC2rev. Sequencing of the PCR product made with primers rpoC2rev identified the presence or absence of the introduced nightshade-specific deletion in rpoC2. Several lines were recovered that had the nightshade-specific deletion in rpoC2. These lines were designated C2A. Others carried the tobacco sequence instead of the nightshade-specific deletion. The aadA cassette was present in all lines. The distance between the insertion/deletion and the aadA cassette is 1320 bp, providing ample space for recombination, as observed in other plastid transformation studies (Kavanagh et al., 1999). These recombinant lines were designated C2N and served as controls during subsequent experiments. Other polymorphic sites tested by PCR with primers EatpAfor/rev and ndtAfor/rev were tobacco-like in all lines, as expected.

Isolation of Nucleic Acids, RNA Analysis, cDNA Synthesis, PCR, and DNA Sequencing

DNA from leaves was isolated as described previously (Doyle and Doyle, 1990). Total leaf RNA was isolated using TRizol reagent (Invitrogen, Carlsbad, CA). RNA gel blots, reverse transcription of RNA primed with hexanucleotide primers using Superscript II (Invitrogen, Carlsbad, CA) amplification of cDNA, and purification of the products were performed as described previously (Schmitz-Linneweber et al., 2001). Sequencing reactions were performed with the DYEnamic Terminator cycle sequenc-

ing ready reaction kit (Amersham–Pharmacia, Buckinghamshire, UK). Sequencing products were analyzed using an ABI PRISM 377 DNA sequencing (Applied Biosystems, Foster City, CA).

Oligonucleotides

The following synthetic oligonucleotides (5’ to 3’) were used for vector construction and analysis of plastid transformants: HatpAfor,
5’-TAGCAAGCTGGTACAATGGCATTGCTC-3’; HatpArev, 5’-TAGCAAGCTGAACCCCTCTCTCTGCAGGCTC-3’; WATfor, 5’-TTAATCTATTATATTGGTCCAAACAAGCGCAAGCTT-3’; CATfor, 5’-TTAATCTATTATATTGGTCCAAACAAGCGCAAGCTT-3’; CATrev, 5’-GAGATCATAAATAGTAGTTAATAAG-3’; Mu-edatpAfor, 5’-CTTATAATATCAAGGCCCAGCCAAACAAGCGCAAGCTT-3’; Mu-edatpArev, 5’-CGGAGCTCGTATATTATTTAAGTAGTGTGTTGTCAGC-3’; EatpAfor, 5’-TTCGCAATTTACCATATCAATCTTATGGTGACGACCTC-3’; EatpArev, 5’-TTCGCAATTTACCATATCAATCTTATGGTGACGACCTC-3’; CGTGAATGC-3’; CGGAGCCGTACAAATG-3’; P3, 5’-AAGGGCCAATTGAATTCCTAAT-3’; P4, 5’-CGGATGATATTAGTAATATTATCC-3’; EatpArev, 5’-GGGGTATAGTTATTGTGAA-3’; rptC2for, 5’-GATTACGAGCCTTAAATACG-3’; HatpArev, 5’-AGGGCCAATTGAATTCCTAAT-3’; ndtAfor, 5’-GGGCAATTTAGAATCTACAGGGT-3’; ndtArev, 5’-GGGCAATTTAGAATCTACAGGGT-3’; CATfor, 5’-AAGCGGATGTAACTCAATCGG-3’; CATrev, 5’-GAGATCATCATAAATGA-3’; EatpAfor, 5’-TTCCAGAATTCTTTCCAAAAGG-3’; WATrev, 5’-TTCCAGAATTCTTTCCAAAAGG-3’; P2, 5’-CTTATAATATACGACGACCCGTCCAA-3’; EatpAfor, 5’-TTCCAGAATTCTTTCCAAAAGG-3’; WATrev, 5’-TTCCAGAATTCTTTCCAAAAGG-3’; P2, 5’-ACTG-3’; WATrev, 5’-TTCCAGAATTCTTTCCAAAAGG-3’. Sequence data from this article have been deposited previously with the EMBL/GenBank data libraries under accession number AJ316582.

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Pigment Deficiency in Nightshade/Tobacco Cybrids Is Caused by the Failure to Edit the Plastid ATPase α-Subunit mRNA

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