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Site-selective inhibition of plastid RNA editing by heat shock and antibiotics: a role for plastid translation in RNA editing

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ABSTRACT

RNA editing in higher plant plastids changes single cytidine residues to uridine through an unknown mechanism. In order to investigate the relation of editing to physiological processes and to other steps in plastid gene expression, we have tested the sensitivity of chloroplast RNA editing to heat shock and antibiotics. We show that heat shock conditions as well as treatment of plants with prokaryotic translational inhibitors can inhibit plastid RNA editing. Surprisingly, this inhibitory effect is confined to a limited number of plastid editing sites suggesting that some site-specific factor(s) but none of the general components of the plastid RNA editing machinery are compromised. Contrary to previous expectations, our results provide evidence for a role of plastid translation in RNA editing.

INTRODUCTION

Plant organellar transcripts can be subject to post-transcriptional pyrimidine-to-pyrimidine conversions referred to as RNA editing. In higher plant plastids, editing involves exclusively cytidine-to-uridine transitions at highly specific sites (1–4). The number of editing sites in the plastid compartment is rather low and was estimated to amount to ~25 sites in the entire 140 kb maize chloroplast genome (5). This low editing frequency markedly contrasts the >1000 editing sites estimated to be encoded in higher plant mitochondrial genomes (6). Otherwise, the two plant organellar RNA editing systems show many similar features and may have originated from common evolutionary roots (3).

Editing in chloroplast transcripts is highly specific and usually very efficient. At most sites, the mRNA population shows a virtually complete transition from C to U. A major question has been how to explain the extraordinarily high specificity of the plastid RNA editing machinery in selecting individual cytosine residues for modification. A number of *in vivo* studies employing chloroplast transformation have demonstrated that, at the mRNA level, sequences immediately flanking the editing site serve as important determinants for the molecular recognition of plastid editing sites (7–10). However, these sequence requirements *in cis* are unlikely to be uniform with respect to their size and their distance from the editing site (8). In addition to the involvement of *cis*-acting elements, there is compelling evidence that site-specific *trans*-acting factors also participate in plastid RNA editing site recognition. Introduction of supernumerary copies of the tobacco *psbL* editing site into the plastid genome by chloroplast transformation has revealed that the editing activity for this particular site is depletable while editing at other sites remained unaffected (7). The participation of site-specific *trans*-acting factors in the editing reactions in chloroplasts was also demonstrated by the rescue of a tobacco plastid RNA editing mutant (created by introducing a heterologous editing site taken from the spinach chloroplast genome which does not undergo editing in tobacco chloroplasts). Editing at the heterologous site could be restored by fusing mutant cells with spinach protoplasts suggesting that a site-specific *trans*-acting factor of extraplastidic origin is required for the editing of this site (11,12). The molecular identity of such *trans*-acting specificity factors for plastid RNA editing has not yet been determined. Also, none of the general (enzymatic?) components of the chloroplast RNA editing machinery have been identified to date.

To gain some information about the chemical nature of the molecules involved in plastid RNA editing (RNA or protein factors?) and their subcellular origin, we have investigated the temperature sensitivity of the editing reaction as well as its sensitivity to prokaryotic translational inhibitors. We show here that elevated temperature and inhibition of chloroplast translation by antibiotics selectively block RNA editing at a small number of sites thus providing a first evidence for a role of the plastid translational apparatus in RNA editing.

MATERIALS AND METHODS

Plant material

Surface-sterilized tobacco (*Nicotiana tabacum* cv. Petit Havana) or barley seeds were germinated on agar-solidified MS medium (13) containing 30 g/l sucrose. Root material was harvested from plants grown in a sterile environment on the same medium. For analysis of heat shock-treated plants, germinating seeds were transferred from 25 to 37°C and kept at this temperature for ~1 week. For experiments with translational inhibitors, surface-sterilized seeds

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were germinated on spectinomycin- or streptomycin-containing medium (500 mg antibiotic/l).

List of oligonucleotides

The following synthetic oligonucleotides were used in this study (I = inosine):

apA1: 5′ CTCCTAGATAAGCTTCACG 3′;
apA2: 5′ GAGCCTCTTCAACAAGGGC 3′;
apF1: 5′ TTTCTTTGGGCCACTGGCC 3′;
apF2: 5′ CCCCTCCACGCAGTTCTTC 3′;
ndi: 5′ CTTGCGGCGCGTATTGTTTGTTGA 3′;
na1: 5′ ACTATTAAATCTTGGGTGG 3′;
ndt1: 5′ GTAAGAGATGTGAATCCGCCTGT 3′;
ndt2: 5′ TGAGTACAGACGTTTCTTTCC 3′;
am3: 5′ GAAGAAAGAAAATTAAGGAACC 3′;
pet1: 5′ TGTATTTCCGGAGTATGAG 3′;
pet2: 5′ CTCTATAAAGGCCCAGAAA 3′;
pet3: 5′ TGTGGGACAATCTACTTTG 3′;
Y: 5′ CCTTCCCTATTCATTGCGGGTTGG 3′;
7652: 5′ CCGAATGAGCTAAGAGAATCTT 3′;
7355: 5′ GACTATAGATCGAACCTATCC 3′;
B5: 5′ ATAATATCAGATTGGGGAGG 3′;
B15: 5′ TGGAATATACCGAATTGTGATC 3′;
B23: 5′ TTGGTIAGATACAATTTGAAGGA 3′;
B24: 5′ TTTIIGTCAATGGATTIGTTTGATC 3′;
nb0: 5′ TGCTTCTCTTCGATGGAAG 3′;
nb2: 5′ GGCGGAACAGATCTACTAATTC 3′;
nb4: 5′ TAATCCTGCATAATCTCGAATG 3′;
nb10: 5′ CAGCTACTCTAGGGGGAATG 3′;
nb11: 5′ TTCATGCTTGTTTGAGTAATAGC 3′;
P12: 5′ TTTTTCTAGAGGTCTAATGAGGCTACTAGG 3′;
P13: 5′ TTTTGGATCCTATACATTTGTGTATTGATA 3′;
P14: 5′ TTTTTCTAGAATATACCAAGAAAGATGTAC 3′;
P16: 5′ TTTTTCTAGACGCTCATATTCATTACCGTA 3′;
P19: 5′ TTTTGGATCCTTTGATGAGAAATAAAAAGG 3′.

Isolation of nucleic acids

Total plant nucleic acids were extracted according to a rapid miniprep procedure described by Doyle and Doyle (14). Total cellular RNA was isolated using the TRIzol reagent (Gibco/BRL). For cDNA synthesis, an aliquot of the RNA preparation was treated with RNase-free DNase I (Boehringer Mannheim).

cDNA synthesis

For cDNA synthesis, total cellular RNA samples were treated with DNase I (Boehringer Mannheim) to remove contaminating DNA molecules. Reverse transcription reactions were primed with random hexanucleotide primers for 10 min at room temperature. The elongation reaction was performed with Moloney murine leukemia virus RNase H-free reverse transcriptase (Gibco/BRL) at 42°C following the manufacturer's instructions.

Polymerase chain reaction (PCR)

DNA and cDNA templates were amplified according to standard protocols (45 s at 94°C, 1.5 min at 55–58°C, 1.5 min at 72°C; 30 or 40 cycles) using a Perkin Elmer GeneAmp 9600 thermocycler. The following primer combinations were employed for amplification of chloroplast DNA and cDNA sequences (see List of oligonucleotides):

ndhA:		ndi/na1;
ndhB	E 0, E I:	P19/nb0;
	E II–E VII:	P14/nb4;
	E VIII:	P16/nb2;
ndhD:		ndt1/ndt2;
atpA:		apA1/apA2;
atpF:		apF1/apF2;
petB:		pet1/pet2;
psbL:		Y/7652;
psbE:		Y/7652;
rpoB:		B23/B24.

DNA sequencing

Direct sequence determination of the PCR-amplified cDNA population or amplified DNA samples was performed by a modified chain termination method as described by Bachmann *et al*. (15). The following synthetic oligonucleotides were used as sequencing primers (see List of oligonucleotides):

ndhA:		na1;
ndhB	E 0:	nb0;
	E I:	nb10;
	E II, E III:	P12, P13;
	E IV, E V:	P16;
	E VI, EVII:	nb11;
	E VIII:	nb2;
ndhD:		am3;
atpA:		apA1;
atpF:		apF2;
petB:		pet3;
psbL:		7355;
psbE:		Y;
rpoB:	E I:	B15;
	E II:	B5.

Hybridization procedures

Total cellular RNA was electrophoresed on formaldehyde-containing 1% agarose gels and transferred onto Hybond N+ membranes (Amersham) using standard protocols (16). A [α-^{32}P]dATP-labelled *ndhB*-specific probe was generated by random priming (Boehringer Mannheim) following the instructions of the manufacturer. Hybridizations were carried out at 65°C in Rapid Hybridization Buffer (Amersham).

RESULTS

Inhibition of plastid RNA editing at two tobacco *ndhB* sites under heat shock conditions

Site-specific *trans*-acting factors have been implicated in the RNA editing reaction in higher plant plastids (7,12). However, the chemical nature of these specificity factors is currently unknown. By analogy to the RNA editing system in trypanosomatid mitochondria (17), small RNA molecules termed guide RNAs could serve as *trans*-acting factors imposing site specificity onto the plastid RNA editing machinery by hybridizing to the mRNA and thereby mediating editing site recognition. If annealing of a small RNA molecule to the mRNA were necessary to allow for editing site recognition in plastids, this first step in the editing

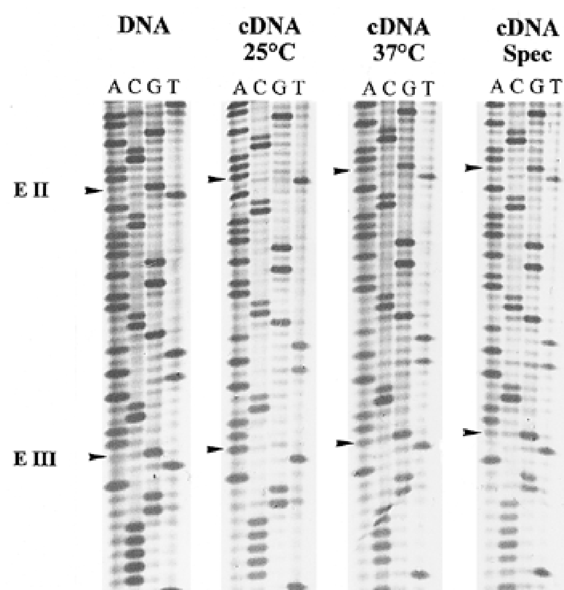

Figure 1. Heat shock and spectinomycin treatment inhibit RNA editing at the *ndhB* sites II and III in tobacco plastids. cDNA sequences are shown for seedlings incubated at 37°C, seedlings germinated on spectinomycin-containing medium (Spec) and for a control sample kept at 25°C without antibiotics. Arrowheads point to the two RNA editing sites. Owing to the polarity of the sequencing primer, the sequence ladders shown here reflect the sequence of the non-coding strand.

reaction should be temperature sensitive and become less efficient at temperatures exceeding the hybridization temperature of the guide RNA and the mRNA template. We therefore set out to test whether elevated temperatures would have an effect on the editing efficiency at chloroplast RNA editing sites.

We transferred tobacco seeds upon germination from standard growth conditions (24–26°C) to 37°C. The seedlings continued to grow at 37°C; the hypocotyl elongated and the apical cotyledon hook opened. However, the plants displayed a pale green phenotype indicating that the heat shock conditions exerted a negative effect on the synthesis or assembly of the photsynthetic protein complexes. After maintenance at 37°C for 7–10 days, the hypocotyls and cotyledons were harvested and used for RNA extraction and cDNA synthesis. We first amplified cDNAs derived from the plastid *ndhB* mRNA since this transcript was previously shown to be highly edited in tobacco and maize (18,19). In addition, it offers the advantage of harboring a group II intron thus allowing for distinguishing cDNA amplification products from the corresponding DNA products by their different sizes. Amplification of cDNA samples from heat shock-treated plants and a control sample (kept at 25°C) using a *ndhB*-specific primer pair (with one primer in each exon) gave rise to products of identical size reflecting correctly spliced transcripts (data not shown). This suggests that plant maintenance at the elevated temperature did not significantly inhibit excision of the *ndhB* intron. We then sequenced directly the amplified cDNA populations using a primer suitable to determine the sequence in the region of *ndhB* editing sites II and III (8). Whereas sites II and III were virtually completely edited in the 25°C control sample, a major portion of the mRNA population turned out to remain unedited in the sample incubated at 37°C (Fig. 1).

In order to test whether plastid editing in general is compromised by elevated temperatures, two other sites in the *ndhB* transcript were analyzed. The editing sites IV and V (8) are located in the same exon of *ndhB* ~100 nucleotides downstream of sites II and III. The amplification products analyzed for site II and III editing also cover sites IV and V thus allowing their direct use for the analysis of editing at the downstream sites IV and V. Interestingly, editing was found to be normal for both site IV and site V in the 37°C sample (Table 1) indicating that heat shock treatment does not exert a negative effect on editing at these two sites. Selective impairment of editing at sites II and III but not sites IV and V may suggest that some site-specific editing factor(s) involved in site II and III editing are either not synthesized or not functional under conditions of elevated temperature.

Table 1. Summary of the effects of heat shock treatment and spectinomycin on editing efficiencies at C to U editing sites encoded in the tobacco plastid genome

Editing site		Control	Heat shock	Spectinomycin	Reference
ndhB	E 0	+ + +	+ + +	+ –	19
	E I	+ + +	+ + +	+ –	
	E II	+ + +	+ –	+ – –	
	E III	+ + +	+ – –	– – –	
	E IV	+ + +	+ + +	+ –	
	E V	+ + +	+ + +	+ + +	
	E VI	+ + +	+ + +	+ + +	
	E VII	+ + +	+ + +	+ + +	
	E VIII	+ + +	+ + +	+ + +	
ndhD		+ –	+ – –	+ – –	30
rpoB	E I	+ + +	+ + +	+ + +	23
	E II	+ + +	+ + +	+ + +	
psbL		+ + +	+ + –	+ –	2
psbE		+ + +	+ + +	+ + –	J. Kudla and R. Bock, unpublished
petB		+ + +	+ + +	+ + +	33,35
ndhA		+ + +	+ + +	+ + +	34
atpA		+ + +	+ + +	+ + +	28
atpF		+ + +	+ + +	+ + +	28

+ + +, virtually complete editing; + + –, partial editing with more edited than unedited molecules; + –, partial editing with about equal proportions of edited and unedited molecules; + – –, partial editing with more unedited than edited molecules; – – –, entirely unedited.

We next wanted to determine the time course of recovery of the *ndhB* site II and III editing activities. We therefore transferred heat shock-treated plants back to standard growth conditions (25°C) and took samples at daily intervals. Analysis of the corresponding *ndhB* cDNA populations revealed that, after only 1 day of incubation at 25°C, editing at both site II and site III was virtually completely restored (data not shown). This fast recovery of the editing activity may indicate that sufficient quantities of the sites II- and III-specific editing factors become rapidly available after lowering the temperature to 25°C.

Inhibition of editing by translational inhibitors

What could be the cause of the drastically reduced editing at *ndhB* sites II and III under heat shock conditions? As discussed above, one possibility is that elevated temperatures could prevent annealing of the site-specific factor(s) to the substrate mRNA. However,

incubation of plants at high temperatures is also known to result in the loss of functional chloroplast ribosomes (20). Thus, the reduced editing at the *ndhB* sites II and III could also be a direct consequence of the absence of chloroplast translation. To distinguish between these two possibilities, tobacco seedlings were grown in the presence of prokaryotic translational inhibitors known to selectively block protein synthesis in the plastid compartment (21). Tobacco seeds still germinate normally in the presence of the aminoglycoside antibiotic spectinomycin at very high concentrations (500 mg/l) expected to lead to a nearly complete block of plastid translation. The seedlings are completely white (22) owing to the lack of chloroplast differentiation. Analysis of *ndhB* editing in these plants revealed an even more dramatic effect on editing at sites II and III. Site II was edited only to a very small extent and site III editing turned out to be completely abolished by spectinomycin (Fig. 1). As a control, again sites IV and V were analyzed. As observed for the heat shock-treated samples, site V was also completely edited in plants grown in the presence of spectinomycin. We observed, however, a small reduction of editing at site IV in the spectinomycin-treated but not in the heat shock-treated plants (Table 1).

Inhibition of editing at *ndhB* sites II and III not only by heat shock treatment but also by spectinomycin strongly suggests that the observed effects are due to the absence of plastid translation. Complete loss of site III editing (and nearly complete loss of site II editing) by spectinomycin treatment as compared with incomplete inhibition by incubation at 37°C may reflect a certain leakiness of the heat shock system, i.e. survival of a small population of plastid ribosomes resulting in some residual translational activity. This conclusion is also supported by the light green phenotype of the heat shock-treated plants as compared with completely white seedlings grown in the presence of the antibiotic.

RNA editing in the *rpoB* transcript of barley plastids was reported earlier to be independent of plastid translation (23). In order to test if inhibition of plastid translation also compromises *ndhB* editing in species other than tobacco we chose barley as a monocotyledonous plant and analyzed *ndhB* editing under heat shock conditions as well as after treatment with prokaryotic translational inhibitors. As numerous other graminaceous plants (24), barley was found to be endogenously resistant to spectinomycin, most probably due to the presence of a spectinomycin-resistant plastid 16S rRNA allele. We therefore tested streptomycin as an alternative antibiotic and bleaching of seedlings germinated on streptomycin-containing medium indicated sensitivity of the barley plastid translational apparatus to this drug. Analysis of *ndhB* site II and III editing revealed that maintenance of barley plants at 37°C nearly completely abolished editing at both sites (Fig. 2). In contrast with the results obtained with tobacco, the inhibitory effect of the antibiotic was smaller than the effect of the heat shock treatment (Fig. 2), most probably due to the delayed action of the drug as caused by its slow uptake by the large barley caryopses. The less severe effect of the antibiotic than that of the elevated temperature is in agreement with the observed incomplete bleaching of the seedlings by streptomycin.

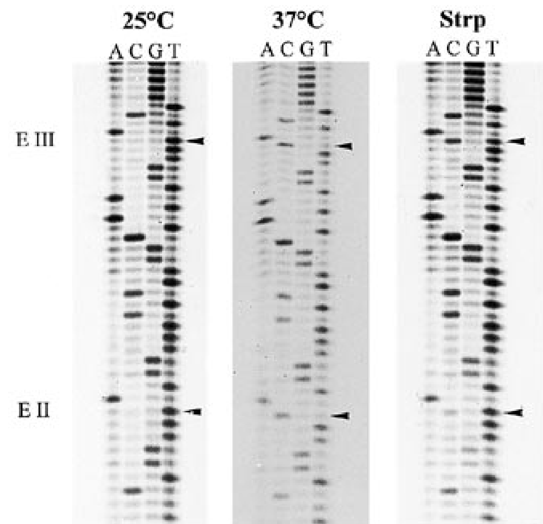

Figure 2. Effect of heat shock and streptomycin treatment on RNA editing at *ndhB* sites II and III in barley plastids. cDNA sequences are shown for seedlings kept at 37°C, seedlings grown in the presence of streptomycin (Strp) and a 25°C control sample. Editing sites are marked by arrowheads. Note that editing at both sites is nearly completely blocked at 37°C, but only partially inhibited by germination on streptomycin-containing medium.

ndhB transcript patterns are not affected by heat shock and antibiotic treatment

Theoretically, the absence of chloroplast translation could exert a negative effect on transcript processing and the inhibited *ndhB* site II and III editing could be a secondary consequence of a defect in one of the other RNA processing steps operating on plastid transcripts. For example, lack of processing of precursor transcripts into mature mRNAs can lead to a drastically reduced editing efficiency as previously observed for a rice mitochondrial transcript (25). To exclude this possibility, we analyzed the *ndhB* transcript patterns upon inhibited plastid translation as compared with a control sample. A major transcript species of ~1.5 kb corresponding to fully processed monocistronic *ndhB* mRNA (32) was detectable in all samples suggesting that the absence of chloroplast translation does not block RNA processing (Fig. 3). This is consistent with the idea that all plastid RNA processing (and degrading) enzymes are probably encoded by nuclear genes. The hybridization detects in addition to the major 1.5 kb species several higher molecular weight mRNAs (Fig. 3) which most likely reflect precursor transcripts and partially processed mRNAs. Their relative abundance is significantly higher in the 37°C and spectinomycin-treated samples than in the 25°C control. However, by no means can this increase account for a complete loss of editing as observed for *ndhB* editing site III in antibiotic-treated plants.

It was recently shown that *ndhB* is transcribed by both plastid RNA polymerases: the plastid genome-encoded *Escherichia coli*-like enzyme and the nuclear-encoded phage polymerase-like enzyme (26). Absence of chloroplast translation is expected to result in a reduced activity of the plastid-encoded polymerase which most likely accounts for the somewhat lower RNA accumulation levels observed in the heat shock- and spectinomycin-treated samples as well as for the differences in relative transcript abundances.

ndhB editing in the absence of chloroplast development

The efficiency of plastid RNA editing can be subject to tissue-specific and developmental stage-specific changes (27–29). Thus, it could be possible that the impairment of editing at the two *ndhB* sites is due to the absence of chloroplast

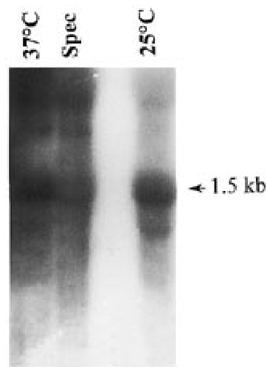

Figure 3. RNA gel blot to compare the tobacco *ndhB* transcript patterns in heat shocked and spectinomycin-treated plants with a control sample kept under standard growth conditions. Hybridization with an *ndhB*-specific probe detects a major 1.5 kb transcript species in all samples corresponding to fully processed monocistronic *ndhB* mRNA (32). Larger transcript species are likely to reflect polycistronic mRNAs synthesized by cotranscription of the *ndhB* reading frame with the upstream ribosomal protein genes *rps12* (exons II and III) and *rps7*.

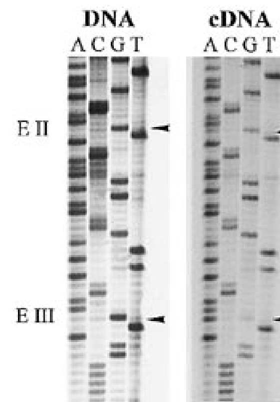

Figure 4. Analysis of *ndhB* editing in the absence of chloroplast development. DNA and cDNA sequences are depicted for tobacco root plastids. Editing sites II and III are denoted by arrowheads. Owing to the polarity of the sequencing primer, the autoradiograph shows the sequence of the non-coding strand. Note that, at site III, where editing is fully inhibited by spectinomycin, editing is independent of the presence of differentiated chloroplasts and the C to U transition is virtually complete also in non-green root plastids. Site II, however, is only partially edited in root plastids suggesting that the decrease in editing efficiency upon block of plastid translation might at least partially be due to the lack of chloroplast development.

differentiation as caused by the inhibition of plastid translation. To exclude this possibility, we analyzed the efficiency of *ndhB* editing in non-green tissue. *ndhB* cDNAs derived from tobacco root RNA were amplified and their editing status was determined by direct sequencing. We found *ndhB* site III (which remained completely unedited upon treatment with spectinomycin) as well as site IV (showing reduced editing in the presence of spectinomycin) fully edited in non-green root plastids indicating that chloroplast differentiation is not required for editing at these sites. Editing at site II (which was partially inhibited by spectinomycin and heat shock treatment), however, was reduced to ~50% in root plastids (Fig. 4) suggesting that the absence of chloroplast differentiation could account for the incomplete editing of *ndhB* site II upon inhibition of plastid translation. This finding indicates that only *ndhB* site II is subjected to plastid differentiation-related changes in editing efficiency and the reduced editing at this site in the absence of plastid translation may thus, at least to a certain extent, be due to the inhibition of chloroplast development. At site III, however, where editing was completely blocked by spectinomycin, the efficiency of editing seems to be independent of chloroplast differentiation.

Sensitivity of editing at other sites to heat shock and antibiotic treatment

In order to test whether the inhibition of editing in the absence of plastid translation is restricted to the analyzed *ndhB* editing sites, we systematically tested all known editing sites encoded in the tobacco chloroplast genome and determined the editing efficiencies under heat shock conditions as well as upon inhibition of protein synthesis by spectinomycin. Editing at most of the sites was still virtually complete in the absence of translation (Table 1). None of the other editing sites encoded in the tobacco chloroplast genome was found to be completely blocked by the loss of plastid translation as observed for *ndhB* editing site III upon plant treatment with spectinomycin. We, however, observed a decrease in editing efficiency for some sites: two other sites in the *ndhB* transcript (sites 0 and I; 19), the *ndhD* initiation codon (30) and two sites in the tetracistronic *psbE/F/L/J* transcript (2; J. Kudla and R. Bock, unpublished; Table 1). As in the case of *ndhB* sites II and III, the inhibitory effect of spectinomycin was more severe than that of the elevated temperature, most likely reflecting a less efficient translation inhibition by heat shock treatment than by the action of the antibiotic. As for sites II, III and IV, we also tested the *ndhB* editing sites 0 and I for whether or not the reduced editing efficiency can be attributed to the lack of chloroplast development. We found both sites to undergo complete editing in root tissue (data not shown) demonstrating that they are efficiently edited also in the absence of photosynthesis and chloroplast differentiation.

DISCUSSION

In the course of this work, we have identified heat shock and plant treatment with prokaryotic translational inhibitors as conditions that inhibit editing at a limited number of plastid RNA editing sites. Block of chloroplast translation by spectinomycin leads to a complete loss of RNA editing at *ndhB* editing site III and reduces editing at some other editing sites encoded in the chloroplast genome (Table 1). Our results establish for the first time, and in contrast with earlier belief (23), a role of plastid translation in the editing of specific sites.

It is well established that certain antibiotics can interact directly with RNA molecules (36). We have, therefore, considered the possibility that the inhibition of plastid editing by spectinomycin or streptomycin is due to a direct binding of antibiotic molecules to the editing sites (or to adjacent mRNA sequences acting as *cis*-elements for editing site recognition). We consider this unlikely since the editing at those sites affected by aminoglycoside antibiotics is also inhibited by heat shock treatment, strongly suggesting that the block of translation is the cause of the inhibited editing reaction.

Amplification of spliced *ndhB* cDNA as well as identity of the transcript patterns in green versus translationally inhibited tissue (Fig. 3) indicate that the loss in editing activity does not reflect a dependence of these editing events on splicing or other RNA

processing steps. We have also considered the possibility that, in the absence of chloroplast translation, *ndhB* transcripts are extremely unstable, thereby not allowing the editing machinery to act efficiently on these substrates. If this were the case, the difference in half-life time of the *ndhB* transcripts would have to be several orders of magnitude, since no editing at all was detected at site III in spectinomycin-treated plants whereas the site undergoes complete editing in untreated control samples. Also, this scenario would involve the additional assumption that editing at the affected sites is much slower than at other sites in the very same transcript (e.g. sites V–VIII which are fully edited in heat shock- and antibiotic-treated plastids). We believe this not to be the case since our transcript analyses failed to provide evidence for substantial degradation of *ndhB* mRNAs in heat shock- and antibiotic-treated plants (Fig. 3).

Analysis of editing in non-green tissue revealed that, except for the reduced editing at site II, the observed effects of inhibited plastid translation on *ndhB* editing cannot be attributed to the absence of chloroplast development. We therefore propose that plastid translation plays a role in editing of some chloroplast RNA editing sites. It was shown earlier that, in the ribosome-free barley mutant albostrians, the plastid *rpoB* transcript is fully edited in spite of the mutant's deficiency in plastid translation (23). In tobacco, we also found *rpoB* editing not affected by blocked plastid translation (Table 1). Inhibition of *ndhB* site III editing in both tobacco and barley and reduction of editing at a number of other sites, however, suggest that plastid RNA editing is not generally independent of translation.

What could be the role that plastid translation plays in editing of those sites? Formally, two possibilities can be envisaged: (i) plastid DNA-encoded gene products are directly involved in the editing reaction by acting as site-specific *trans*-acting factor(s) for the molecular recognition of some editing sites or (ii) plastid translation product(s) serve as auxiliary factor(s) mediating or improving the accessibility of the substrate site to the editosome. We favor the latter possibility since it provides a more plausible explanation for the fact that, except for *ndhB* site III where editing is completely blocked in the absence of translation, editing is incompletely inhibited in the other cases. At present, we can only speculate about which plastid genome-encoded gene product(s) could be involved in the editing of these sites. Proteins with RNA-binding capacity, such as ribosomal proteins, would be attractive candidates. Alternatively, active translation of the transcript by functional chloroplast ribosomes might resolve mRNA secondary structures that otherwise would disturb the accessibility of the editing site to the plastid RNA editing machinery.

In view of the many similarities between the RNA editing processes in chloroplasts and plant mitochondria, it would be interesting to test the plant mitochondrial RNA editing system for its sensitivity to heat shock and translational inhibitors. However, nothing is currently known about the sensitivity of the plant mitochondrial translational apparatus to elevated temperatures or antibiotics. None of the many spontaneous antibiotic resistance mutations characterized thus far appears to reside in the plant mitochondrial genome (31) suggesting that the plant mitochondrial translational apparatus may be resistant to at least some of the classical prokaryotic inhibitors of protein biosynthesis.

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