Analysis of silent RNA editing sites in atp6 transcripts of Sorghum bicolor

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Abstract We have observed numerous examples of silent or rare non-silent editing sites in the amino-extension and part of the conserved core of mitochondrial atp6 transcripts of Sorghum. In this region of the 1.4-kb atp6-2 mRNA (position 300 to 550) two editing sites, which alter the amino-acid sequence and occur in all cDNAs analysed, were already known, while nine others were found which are silent or occur in a few mRNAs only. Many aspects of RNA editing in the mitochondria of higher plants are still unknown. This includes the influence of genomic background or silent RNA editing. We were interested in the influence of nuclear and mitochondrial backgrounds on RNA editing. Previous preliminary results indicated the possibility of line-specific editing at silent sites. However, a more comprehensive approach gave no consistent evidence for such editing. These results are discussed with respect to their potential impact on the evolution of mitochondrial genes.

Key words atp6 · Silent RNA editing · Sorghum bicolor

Introduction

A number of mechanistic types of RNA editing are known, including trypanosome and higher-plant RNA editing (reviewed in: Cattaneo 1991, 1992). In higher plants, RNA editing is known from both mitochondrial and chloroplast transcripts (reviewed in Gray et al. 1992; Pring et al. 1993), although the majority of known RNA editing sites has been found in mitochondrial transcripts. Usually C-to-U editing is observed, with rare U-to-C edits (Gualberto et al. 1990; Schuster et al. 1990). Most of the editing sites reported result in changes of the amino-acid sequence as deduced from the genomic DNA sequence. Those changes restore sequences conserved among fungal and animal mitochondrial genes (reviewed in Pring et al. 1993). In addition, silent editing, which does not change amino-acid sequences, was occasionally reported (e.g. Kempken et al. 1991; Schuster and Brennicke 1991). Finally, rare cases of RNA editing include the generation of premature stop codons (Schuster and Brennicke 1991; Sutton et al. 1993).

We previously observed variation in the frequency of silent editing between the S. Sorghum lines Tx398 and IS1112C (Kempken et al. 1991). In the present study we expand these observations to include male-sterile and fertility restored versions of the IS1112C cytoplasm, which allows an assessment of editing frequency as influenced by nuclear background. We chose a 250-bp region, including part of the N-terminal extension of the atp6 open reading frame (Kempken et al. 1991; Mullen et al. 1992). In addition to two non-silent sites edited in 100% of the clones examined, four silent sites with variable frequency were observed, and five sites were found that occurred only once in 51 clones. Cumulatively our data provide only suggestive evidence for nuclear-background effects on silent-editing frequency.

Materials and methods

Sorghum lines and culture. Four sorghum lines were used in this study: TX398, a normal cytoplasm line; IS1112C, a fertile line carrying a male-sterile cytoplasm; A3TX398, a male-sterile line carrying the IS1112C cytoplasm (Mullen et al. 1992), and an F1, inc. A3TX398 represents a near-isogenic line carrying the IS1112C cytoplasm in a TX398 background; this line had been backcrossed at least ten generations. The F1 was produced by three selfed generations of the F1 of the cross A3TX398×IS1112C. Seedlings were grown at 22 or 28 °C in the dark for 7 days prior to harvest.

E. coli strain and culture. The E. coli strain used for DNA cloning was XLBlueI (Stratagene), with standard culture conditions (Sambrook et al. 1989).
Results and discussion

Genomic and cDNA sequences of the *Sorghum* *atp6-2* copy have been reported previously (Kempken et al. 1991; Mullen et al. 1992). In all the *Sorghum* lines used a 1.4-kb transcript at roughly equal abundance was detected, which corresponds to the main transcript of the *atp6-2* copy found in all *Sorghum* lines (Mullen et al. 1992). No influence of either cytoplasm or nuclear background on transcript abundance was observed, as was reported in petunia. There, a correlation of nuclear background-influenced transcript abundance and RNA editing was described (Lu and Hanson 1992). Reverse transcription of DNAse-treated total RNA with oligonucleotide FK52 and subsequent PCR amplification with oligonucleotides FK30 and FK52 exhibited a modified restriction pattern, if RNA editing has occurred. Numbering starts at the 5' end of the 1.4-kb *atp6-2* transcript. A 41-bp fragment found exclusively in edited cDNAs is marked

RNA-isolation and Northern blotting. Total RNA was isolated from 5 to 10 g of etiolated seedlings, using the method of Mohr et al. (1993). Total RNA was separated on 1.2% agarose gels containing formaldehyde (Sambrook et al. 1989). Northern blotting was performed employing a Posi-Blotter (Stratagene).

RT-PCR amplification, restriction analysis, cloning, and sequencing procedures. Reverse transcription and PCR amplification (RT-PCR) was essentially as described in Kempken et al. (1991). Oligonucleotides were 5'CGATGCCCCCGGCGCCGATACC (FK30) and YTGACCGGCCATCATATTAGC (FK52). For oligonucleotide localisation see Fig. 1. All sequencing reactions were done employing oligonucleotide FK31 5'CCAGGTTCGGCACGAAATCA or FK30 (see above). To perform restriction analysis of amplified cDNAs, PCR reactions were purified with a PCR-spin-column kit (Qiagen). Restriction analysis was carried out as suggested by the manufacturer (Boehringer). Vector pBluescriptII+ (Stratagene) was used for the cloning of cDNAs. All cloning procedures were as described (Sambrook et al. 1989). Sequencing of cloned cDNAs was performed with a Sequenase-2.0 kit (USB).