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Abstract

The artificial expression of antisense RNA is commonly used in eucaryotes, especially higher plants, to reduce the level of specific proteins (van der Krol et al. 1988 Nature 333:866-869). Here we report the use of antisense RNA to inhibit the translation of a subunit of the mitochondrial NADH:ubiquinone oxidoreductase, the respiratory complex I in *N. crassa*

An antisense RNA expression vector for *Neurospora crassa*

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The artificial expression of antisense RNA is commonly used in eucaryotes, especially higher plants, to reduce the level of specific proteins (van der Krol et al. 1988 Nature 333:866-869). Here we report the use of antisense RNA to inhibit the translation of a subunit of the mitochondrial NADH:ubiquinone oxidoreductase, the respiratory complex I in *N. crassa*.

Fragments from the quinic acid inducible *qa* gene cluster (Geever et al. 1989 J. Mol. Biol. 207:15-34) were used to construct an antisense RNA expression vector (Figure 1). A full-length cDNA of the 51 kDa NADH binding subunit of complex I (Weiss et al. 1991 Eur. J. Biochem. 197:563-576) was inserted in reverse orientation between a promoter frag-ment of the *qa-2* gene, including four regulation sites, and a polyadenylation and termination fragment of the *qa-4* gene. The *am* gene was used as a selectable marker for transformation of *am* deficient mutants (Kinsey and Rambosek 1984 Mol. Cell. Biol. 4:117-122)

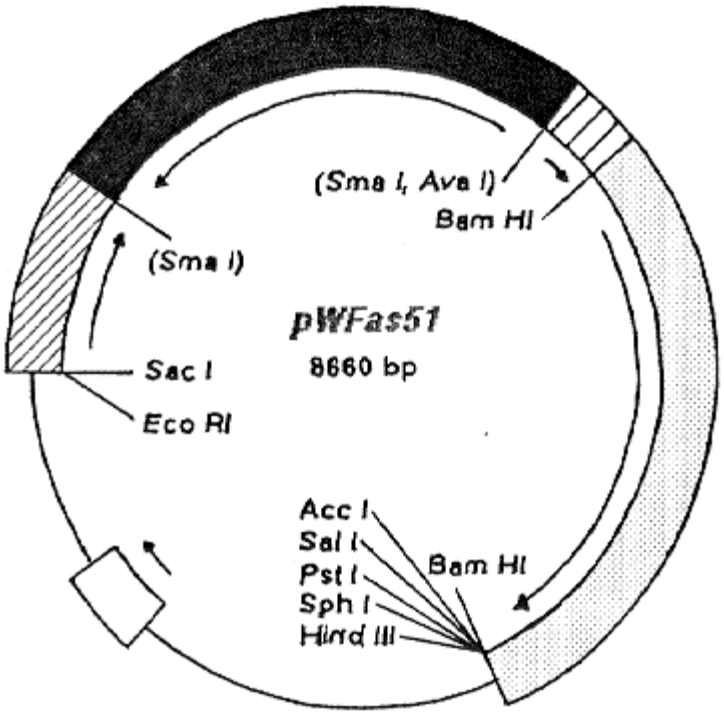

Figure 1. Construction of the antisense RNA expression vector pWAFas51. A 380 bp *Bam*HI/*Ava*I fragment of the *qa-4* gene, which includes regions for polyadenylation and termination of the *qa-4* RNA, was inserted into the multiple cloning site of the parental pT7T3 (Pharmacia) by destroying the AvaI site. A 980 bp *Sac*I/*Sma*I fragment of the *qa-2* gene with the promoter region and four regulation sites - for induction with quinic acid - was also inserted in

the MCS. A 2600 bp *Bam*HI fragment of the *am* gene was used as a selectable marker and ligated into the *Bam*HI site of the vector. The recessed 3' ends of the 1800 bp cDNA of the *51 kDa* gene were filled in and the blunt-ended molecules were ligated in reverse orientation into the *Sma*I site of the RNA expression vector; the *Sma*I site was destroyed by this step. The open box represents the ampicillin resistance gene while the dark-slashed and light-slashed boxes indicate the *qa-2* and *qa-4* areas respectively. The filled box represents the cDNA of the *51 kDa* gene and the stippled box shows the *am* gene. The arrows indicate the direction of transcription.

After transformation, the integration of the plasmid into the genome was detected by Southern blot analysis. In heterokaryotic transformants grown with quinic acid as sole carbon source, the antisense RNA was expressed (Figure 2) leading to 50 to 80% reduction of the translation of the subunit (Figure 3), depending on the time of incubation with quinic acid. No difference in the level of transcript from the endogenous *51 kDa* gene could be ascertained between transformants grown with quinic acid nor with sucrose as sole carbon source. Transformants grown with sucrose showed no reduction of the 51 kDa protein and only a slight expression of antisense RNA. The transformants showed no defects in mitochondrial respiration (see also Nehls et al. 1992 J. Mol. Biol. 227:1032-1042).

The described technique is superior to other methods (such as gene disruption) whenever the complete loss of a protein has lethal consequences for the organism. The inducible RNA expression vector without the am gene and the *51 kDa* gene (named pWF1) is available in the Fungal Genetics Stock Center. Each cDNA to be transcribed can be blunt-ligated into the SmaI site of the vector, in sense or antisense orientation. The unique restriction sites *Eco*RI, *Bam*HI, *Acc*I, *Sal*I, *Pst*I, *Sph*I and *Hin*dIII can be used for ligating different *N. crassa* selectable transformation markers into the vector.

The financial support of the Deutsche Forschungsgemeinschaft is acknowledged.

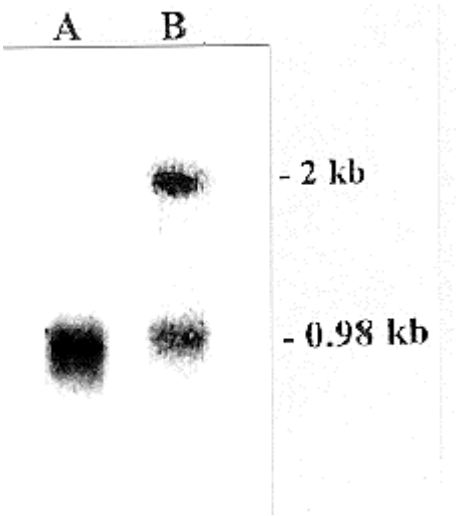

Figure 2. Northern blot analysis of antisense RNA expression in mutant (lane B) and wild type (lane A) cells. Total RNA was isolated, subjected to electrophoresis in a formaldehyde agarose gel and transferred to a nitrocellulose membrane. The RNA was hybridized with in vitro labelled transcripts from the *51 kDa* gene (in the sense orientation). As a control, an in vitro labelled

antisense transcript from the *21.3 kDa* gene, which encodes a subunit of the membrane arm of complex I, was included in the hybridization. A signal with the expected 2 kb length of the antisense RNA for the 51 kDa protein could be seen only in the transformant RNA (lane B), while both wild type and transformant RNAs have a 0.98 kb transcript which hybridized with the control probes for the 21.3 kDa protein.

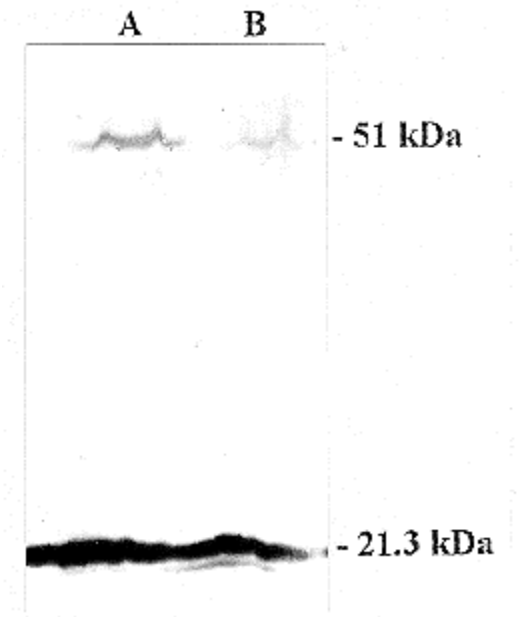

Figure 3. Western blot analysis of one mutant (lane B) compared with the wild type (lane A). The same amount of mitochondrial protein was incubated with antisera against the 51 kDa subunit and, as a control, against the 21.3 kDa subunit of the membrane arm of complex I. The level of 51 kDa protein in the mutant is reduced by 80% compared to the wild type.