

## Cloning of an Additional cDNA for the Alternative Oxidase in Tobacco

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The alternative oxidase is a cyanide-insensitive terminal oxidase found in a variety of organisms although it is best characterized in plants. It branches from the Cyt chain at the level of ubiquinone and does not pump protons and is thus non-energy-conserving. In thermogenic floral appendages it plays a clear role in the volatilization of compounds to attract insects for pollination, but its role in nonthermogenic plants is unclear (Moore and Siedow, 1991). Induction of the alternative oxidase at the gene level has been characterized in a number of studies with compounds that inhibit the Cyt chain. Additionally, aging of potato slices, treatment with ethylene in fruits and storage tissue, cold treatment in tobacco (*Nicotiana tabacum*) and wheat, and salicylic acid treatment of *Sauromatum guttatum* have all been shown to induce the alternative oxidase (Day et al., 1995). At the biochemical level allosteric stimulation by pyruvate and the oxidation reduction state of the protein have been shown to be important determinants of activity (Millar et al., 1993; Umbach and Siedow, 1993).

We have isolated and sequenced a cDNA clone from tobacco for the alternative oxidase. The predicted protein shows high identity with alternative oxidase from other species (Table I). However, when it was compared with the recently published sequence from tobacco (Vanlerberghe and McIntosh 1994), it showed significant differences, having 93 and 95% identity at the nucleic acid and protein levels, respectively (Vanlerberghe and McIntosh, 1994). The gene sequenced in this study is 209 bp shorter at the 5' end compared to that of Vanlerberghe and McIntosh (1994); additionally, there are 47 base pair differences in the open reading frame, which translate into 14 differences in amino acids. At the 3' end there are 13 base pairs different between the two clones.

The differences between these two tobacco clones may be accounted for by varietal differences between the two studies, since we used cv SR1 versus Bright Yellow, which was used by Vanlerberghe and McIntosh (1994). Alternatively, the differences in sequence may indicate a second gene for the alternative oxidase in tobacco. This possibility is supported by the following: (a) *N. tabacum*

**Table I.** Alternative oxidase cDNA clone from tobacco

Organism:	<i>Nicotiana tabacum</i> var SR1.
Function:	Cyanide-insensitive terminal oxidase from mitochondria.
Genome Localization:	Nuclear (determined by Southern analysis).
Cloning Techniques:	A 1.13-kb cDNA clone was isolated from a tobacco shoot library (Stratagene) using a soybean alternative oxidase cDNA as a probe (Whelan et al., 1993).
Method of Identification:	High homology with other alternative oxidase sequences.
Percent Identity of Predicted Protein to Other Alternative Oxidase Proteins:	Tobacco, 95; soybean, 79; Arabidopsis, 78; <i>S. guttatum</i> , 74; <i>Hansenula</i> , 32.
Predicted Protein Sequence:	The predicted protein is 297 amino acids with a putative mitochondrial targeting peptide of 13 amino acids. The precursor protein has a predicted molecular mass of 34 kD and the mature protein has a predicted molecular mass of 32.5 kD.
Subcellular Localization:	Mitochondrial inner membrane.

is an amphidiploid plant that arose from two progenitor species and it has been shown for the Rieske FeS protein that genes have come from each parental species (Huang et al., 1994); (b) the 5' ends of the two clones are significantly different in length; thus, although the predicted mature proteins from both genes will have the predicted molecular mass of 32.5 kD, the precursor proteins will have predicted molecular masses of 34 kD (this study) and 40 kD (Vanlerberghe and McIntosh, 1994); (c) Southern analysis suggests two different genes but cannot be conclusive because the genomic structure of the gene(s) are unknown; (d) sequence analysis of cDNA clones from different soybean varieties show only 7 base pairs difference and no structural differences. Therefore, we suggest that tobacco possesses two copies of the *Aox* gene and suggest that these be termed *Aox1a* (Vanlerberghe and McIntosh, 1994) and *Aox1b* (this study).

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