Characterization of the multifunctional carbonic anhydrase/ NADH-dehydrogenase complex of plant mitochondria

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Recently, plant specific subunits of complex I were identified by mass spectrometry, which exhibit sequence similarity to a archaebacterial γ-carbonic anhydrase (CA) (**Fig. 1**). A project was started to functionally characterize these proteins by the use of Arabidopsis knock out plants.

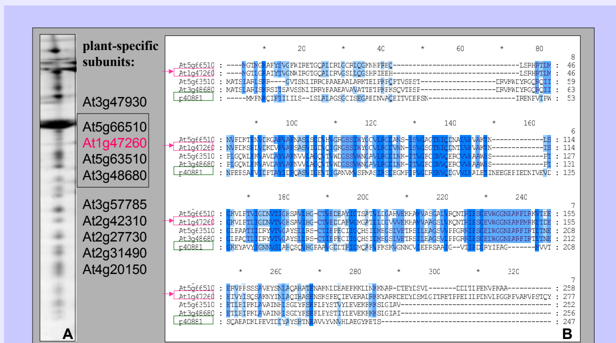

Fig. 1 A: Arabidopsis complex I separated by 2D blue-native / SDS-PAGE. B: Sequence alignment of four plant-specific subunits of complex I with *M. thermophila* carbonic anhydrase (**p40881**). Amino acids conserved in at least four sequences are underlayed in blue.

A homozygous knock-out line for one of these putative carbonic anhydrase subunits of complex I (At1g47260) did not show any altered phenotype if compared with wild-type Arabidopsis plants (**Fig. 2A**). In contrast, a suspension cell culture generated form mutant plants showed a clearly reduced growth rate and reduced oxygen consumption (**Fig. 2B**).

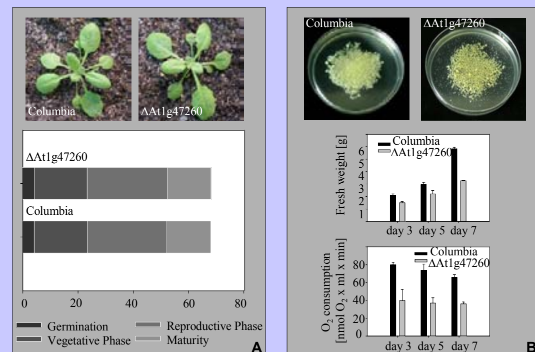

Fig. 2 A: Phenotyp and development of wild-type versus mutant plants B: Phenotyp, growth rate and O_2-consumption of wild-type vesus mutant cell culture

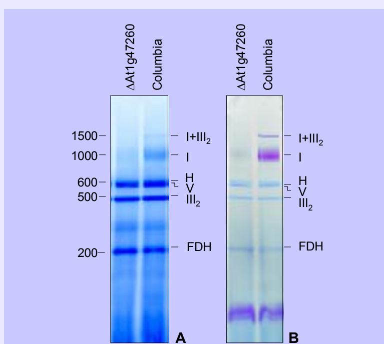

Fig. 3 Separation of total mitochondrial protein by 1D blue-native PAGE. A: Coomassie-colloidal stained gel, B: complex I activity stained gel. I-V = respiratory complexes I-V, I+III$_2$: supercomplex formed by complexes I and III$_2$, H = HSP60, FDH = format dehydrogenase

Separation of the respiratory chain complexes by 1D blue-native PAGE revealed that the knockout of At1g 47260 leads to a drastic reduction of complex I **(Fig. 3)**. This observation was confirmed by *in-gel* activity staining for complex I.

To address the question whether complex I reduction is due to reduced stability of complex I during blue-native PAGE, or an *in vivo* consequence of the *knockout*, a 2D fluorescence DIGE experiment was carried out (**Fig. 4A**) This method allows to compare the amount of complex I subunits in mutant and wt cells in one single gel. Indeed complex I subunits are truly reduced in the mutant cells (green spots indicated by white arrows in **Fig, 4B**).

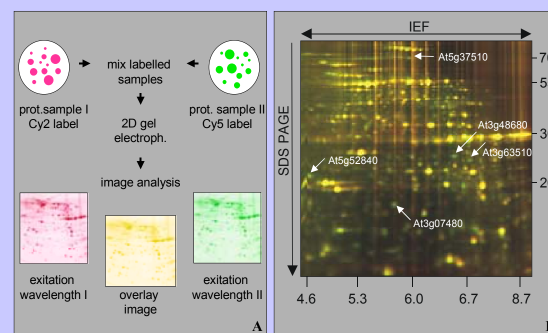

Fig. 4 A: Principle of 2D fluorescence differential gel electrophoresis (DIGE). B: 2D IEF/SDS-PAGE with total mitochondrial protein of mutant (red) and wt (green) cells. Proteins of equal amount in mutant and wt cells are yellow, proteins reduced in mutants green and increased in red. White arrows indicate complex I subunits.

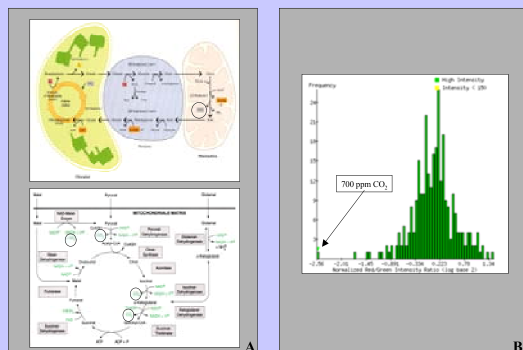

Fig. 5 A: Photorespiration and malic enzyme as additional sources for CO_2 in plant mitochondria. B: Decreased abundance (-2.56 fold) of At1g47260 mRNA at elevated CO_2-levels**.** Imag. in Fig. 5A taken from Heldt (2003), Raven et al. 2000.

In conclusion, At1g47260 most likely is involved in primary carbon metabolism. However, why do plant mitochondria need this additional enzymic activity, which is absent in mitochondria from animals and fungi? An explanation could be the large extend of CO_2 liberation during photorespiration and direct conversion of malate into pyruvate in plant mitochondria.

References:

Heazlewood et al. 2003, BBA 2604, 159-169, Millar et al. 2003, Plant Physiol. 133, 443-447
Heldt, H. (2003) Pflanzenbiochemie Spektrum Verlag, Heidelberg, p. 145
Perales et al. (2004) manuscript in preparation

Raven, P., Evert, R. und Eichhorn, S. (2000) Biologie der Pflanzen. W. de Gruyter, Berlin, p156
http://genome-www5.stanford.edu/cgi-bin/scriptIndex.pl