Characterization of putative acetate transporters in *Chlamydomonas reinhardtii*

Lorenzo Durante[1], Marc Hanikenne[2], Claire Remacle[1]

[1]Lab of Genetics and Physiology of Microalgae – [2]Functional Genomics and Plant Molecular Imaging
Université de Liège, Belgium

Introduction

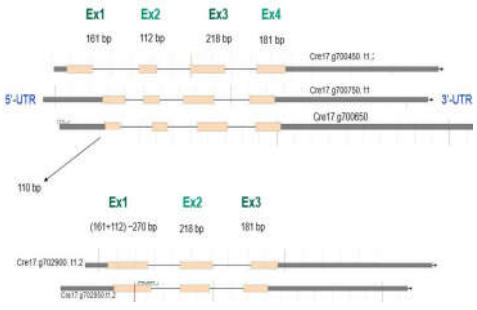

Fig. 1 - Structure of genes

The unicellular green alga *C. reinhardtii* can grown either phototrophically with CO_2 as the sole carbon source, heterotrophically by consuming acetate in the dark and mixotrophically by using both carbon sources in the light. Despite significant knowledge gained on acetate metabolism, the genes coding for acetate transporter/permease are still unknown in this alga. However, recent analyses[1,2] have shown five functionally uncharacterized members of the **GPR1/FUN34/yaaH (GFY)**, a protein family which includes genes involved in carboxylic organic acid uptake/sensing already described in bacteria, yeasts and filamentous fungi. Thus, the five genes identified in *C. reinhardtii* as Cre17.g700450 (GFY1), Cre17.g.700650 (GFY2), Cre17.g.700750 (GFY3), Cre17.g.702900 (GFY4) and Cre17.g.702950 (GFY5) encode for putative acetate transporter proteins given that they are structured in 6-7 hydrophobic transmembrane helices. They are characterized by a close gene structure (Fig. 1) and very high similarity in their coding sequence (CDS) except for a clear distinction at the N-terminus amino acid sequences (Fig. 2).

A reverse functional genomics approach by using artificial micro RNA (amiRNA) gene silencing was adopted to target the five genes one-by-one. Until now, ~160 transformants were generated for each amiRNA construct and their characterization is ongoing. A further characterization of the mutants will follow to have an understanding of the gene function in the acetate metabolism.

Fig. 2 – CDS amino acidic sequence alignment
yellow square highlights N-terminus differences

Results

Transcripts quantification

In particular, we showed that GFY1 expression was slightly diminished in presence of acetate, GFY2 and GFY3 transcripts were not varying in presence or absence of acetate, suggesting a constitutive expression. On the other hand, GFY5 and GFY4 were specifically highly expressed in the dark, or low light condition but not anoxia. Interestingly, GFY5, and to less extent, display expression that seems reversed to the light intensity used.

Almost all transcripts were downregulated when cells were exposed to high light. GFY1 and GFY2 were especially expressed in anoxic condition were normally acetate is excreted outside the cell. In conclusion, our preliminary data suggest differentiated roles of the acetate transporters into acetate metabolism and/or eventually a different subcellular localization.

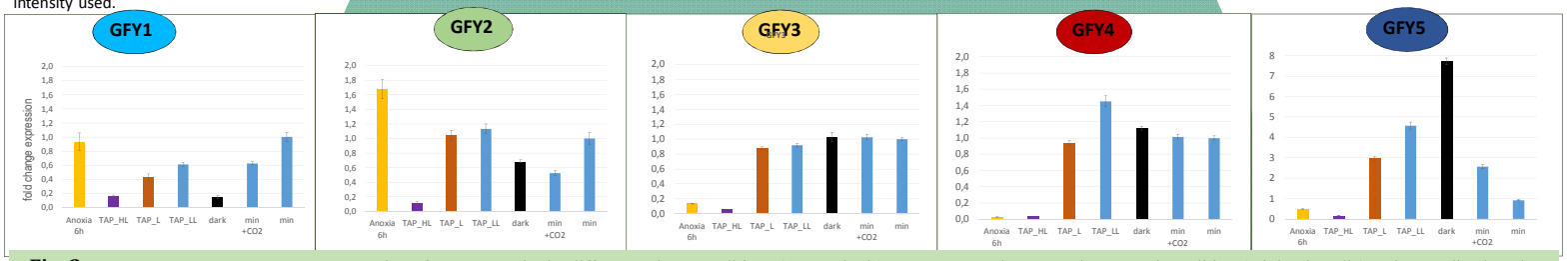

Fig. 3 - Analysis of real-time qPCR: expression of GFY genes in the different culture conditions (see Methods) were expressed compared to control condition (minimal media) and normalized on the basis of two reference genes (BTUB II, CBLP). Reproducibility was guarantee by technical sample triplicates.

Co-expression

GFY genes display a strictly correlation with the expression of genes involved in carbon metabolism, especially related to the primary biochemical steps of acetate assimilation, *i.e.* acetyl-CoA synthase, acetate kinase, isocitrate lyase, malate synthase and malate dehydrogenase etc..

Methods

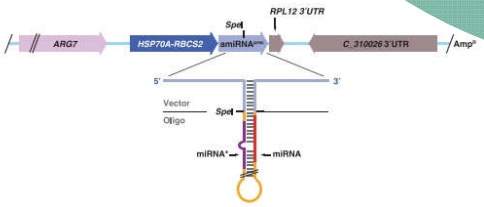

Fig. 4 - amiRNA vector: selection markers: ARG7 and Amp^R

Fig. 5 - Primer design: strategy adopted to obtain gene-spefic primers due to the high similarity inside the CDS (see Fig. 2)

Cells of wall-less strain 325.3 (wt-137 mt+) were cultivated in six different conditions:

TAP_L Control condition (acetate 16.65 mM, 60 uE m^{-2} s^{-1} PAR)

TMP_L Autotrophy (minimal medium, 60 uE m^{-2} s^{-1} PAR)

TAP_LL Low light (acetate 16.65 mM, 15 uE m^{-2} s^{-1} PAR)

TAP_HL High light (acetate 16.65 mM, 260 uE m^{-2} s^{-1} PAR)

TAP_D Hetrotrophy (acetate 16.65 mM, dark)

Anaerobiosis 6h of anaerobic adaptation by N2 purging

Conclusion

The GPR1/FUN34/yaaH genes found in *C. reinhardtii* are characterized by very high similarity in their coding sequences, letting us to think for a redundant role. However, the divergent amino acid composition at the N-terminus and the distinct expression under the different culture conditions tested point to a different situation. In conclusion, our preliminary data suggest differentiated roles of the putative acetate transporters into acetate metabolism and/or eventually a different subcellular localization.

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References:
[1] Goodenough *et al.* (2014) Eukaryotic Cell, 13: 591-613
[2] Merchant SS *et al.* (2007) Science, 318: 245–250