Characterization of putative acetate transporters in *Chlamydomonas reinhardtii*

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Introduction

The unicellular green alga *C. reinhardtii* can grown 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 GFY1-5 encode for putative acetate transporter proteins given that they are structured in 6 hydrophobic transmembrane helices. They are characterized by a close gene structure (Fig. 1) and very high similarity in their coding sequence (CDS) except at the N-terminus amino acid sequences (Fig. 2). Insertional mutants for the genes GFY1, 2 and 3 are available, and artificial microRNA (amiRNA) technique will be used to generate knock-down mutant for GFY4, 5 and all the 5 genes. Mutants will be used to investigate about the role of this putative acetate transporters by placing them in different culture conditions. Plus, protein localization experiments will already give some clues about a putative peroxisomal localization (Fig. 3). If confirmed, as far as we know this work could represent the first attempt to describe acetate transporters in this microalga.

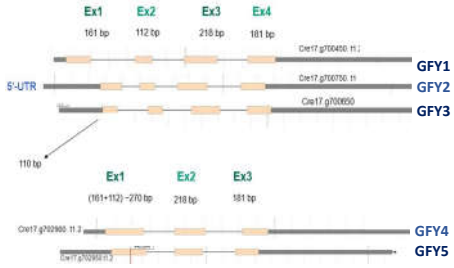
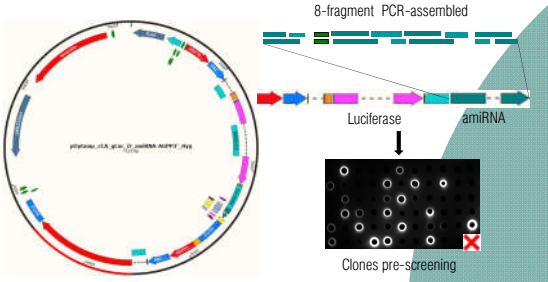


Fig. 1 – Gene organisation into the genome

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

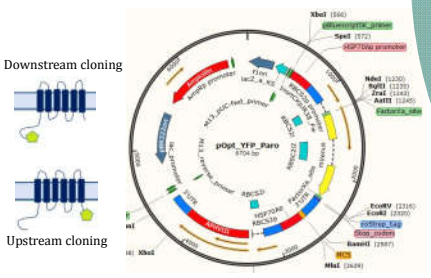
Material & Methods

artificial microRNA silencing



pOpt_cCA_gLuc_amiRNA

NEW SEED SEQUENCES TO TEST !



pOpt_YFP (mVenus)

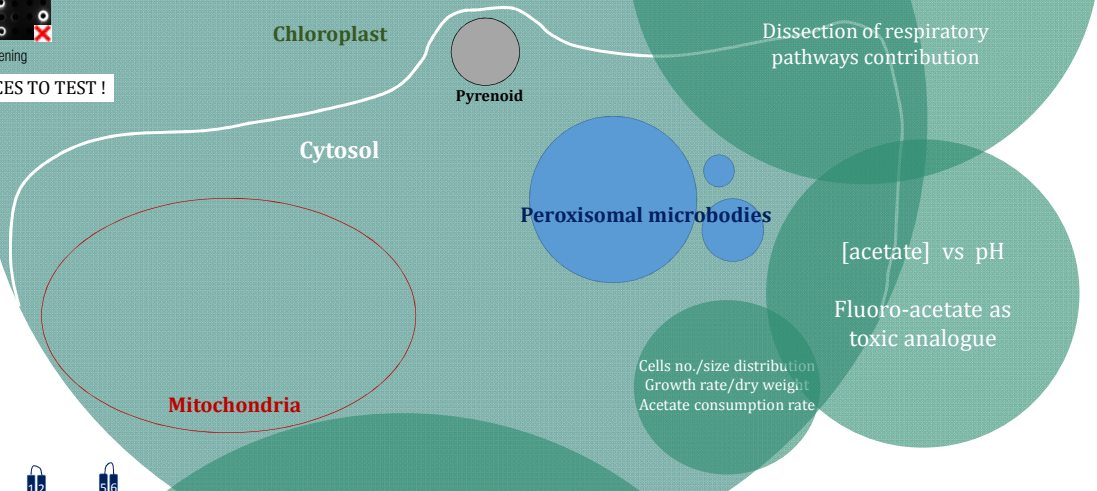
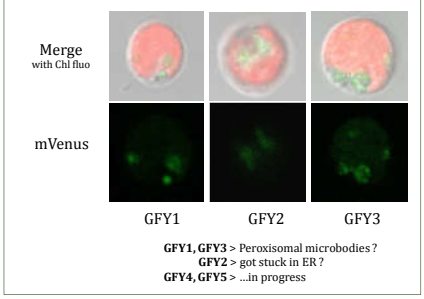
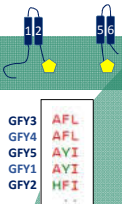


Fig. 3 Protein localization



GFY1, GFY3 > Peroxisomal microbodies ?
GFY2 > got stuck in ER ?
GFY4, GFY5 > ...in progress

Results



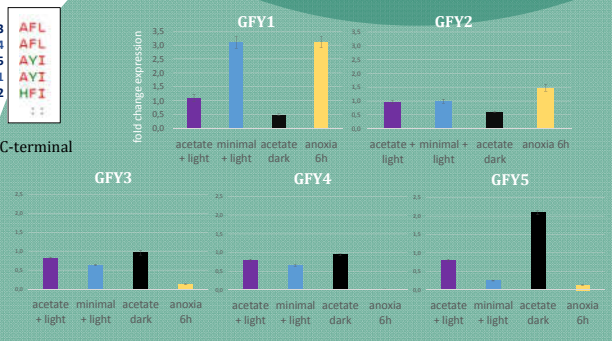


Fig. 3 – Expression level qRT-PCR: $2^{-\Delta\Delta Ct}$ normalization (CBLP/RPL13/BTUB)

Gene expression

The divergent N-terminus sequences and the distinct **expression pattern** in different cultivation conditions tested, point to a different situation. In particular, our qRT-PCR analyses showed that ***GFY1* and *GFY2* transcripts were more abundant in anaerobiosis** while ***GFY3*, *GFY4*, and *GFY5* were mainly expressed during acetate assimilation**. In support of these findings, associated co-expressed genes also exhibited similar expression patterns, typical of each condition,

Conclusion

At first glance, GPR1/FUN34/yaaH genes found in *C. reinhardtii* seem to derive from gene redundancy. However, the N-terminal divergent amino acid composition and the distinct expression under the different culture conditions tested point to a different situation. Indeed, our preliminary data suggest two differentiated expression patterns, one co-expressed with fermentation pathway (GFY1, GFY2) and the second that match with acetate assimilation metabolism (GFY3, GFY4, GFY5). This putative acetate transporters will show eventually a different subcellular localization, from the cellular membrane (GFY2) to the inner membrane of peroxisomes (GFY1, 3, 4 and 5).

fnrs L. Durante is recipient of FRIA (Fonds pour la Recherche Industrielle et Appliquée) fellowship.

1 Goodenough *et al*. (2014) Eukaryotic Cell, 13: 591-613
2 Merchant SS *et al*. (2007) Science, 318: 245–250
3 Lauersen K J *et al*. (2013, 2015, 2016)