The GPR1/FUN34/YaaH(/SatP) members in *Chlamydomonas reinhardtii*, are they novel peroxisomal transporters?

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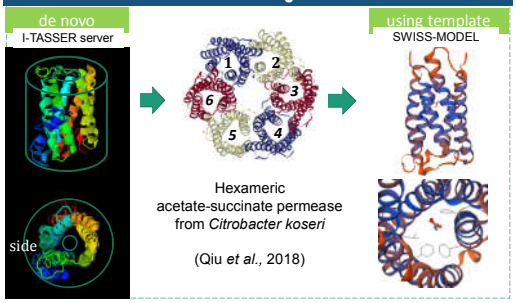
Abstract

The green microalga *C. reinhardtii* can grow heterotrophically in presence of acetate in the dark as sole carbon source, due to the anaplerotic pathway of glyoxylate cycle[1] almost completely localised in the peroxisomal microbodies[2] . Despite the significant advances gained on carbon metabolism, the genes involved in acetate transport are not yet identified. Recently, five functionally uncharacterized members predicted to have transporter-like structure were classified within the protein family GPR1/FUN34/YaaH(/SatP) (GFY)[3], which comprises genes coding for putative carboxylic acids transporters in other microorganisms, including acetate as substrate[4]. In *C. reinhardtii, GFY1-5* as we shown by YFP fusion protein, localise to peroxisomal membranes. In addition, we also showed that these genes present distinct expression patterns in different cultivation conditions, which correlates with associated co-expressed genes found in the databases. Therefore, although these proteins share between 78 and 97% similarity between their amino acid sequences, this might suggest non-redundant roles. To gain insight on their role, we are currently analysing insertional and knock-down mutants. Mutants for the genes *GFY1*, *GFY2* and *GFY3* were obtained from the *Chlamydomonas* Library Project [5]. Mutants for *GFY4*, *GFY5* and all *GFY* genes are presently under generation via artificial micro RNA. In conclusion, GFY members could represent different protagonists in transporting substrates in/out of peroxisomal microbodies, as putative transporters/permeases function.

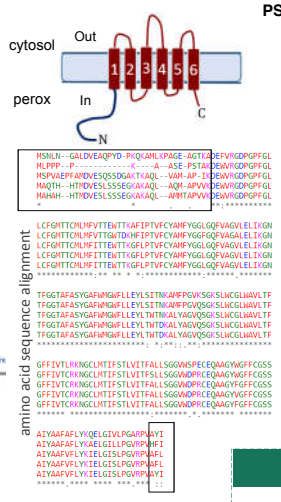
In silico analysis

- GFY proteins have been described as **organic acids permease** in yeast, fungi and bacteria.
- Amino acid signature doesn't correspond to **any functional domain**
- Secondary structure predictions reveal **6 transmembrane helix** proteins
- Putative **PEX19 binding site** and weak Peroxisomal Target Signal type 1 (**PTS1**)

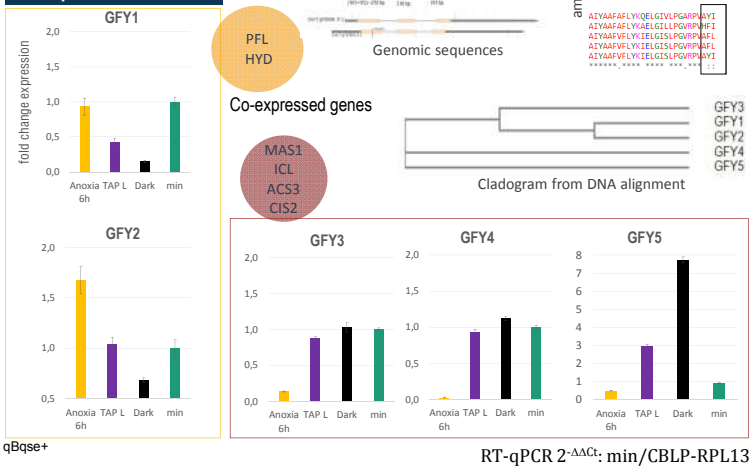
Modeling



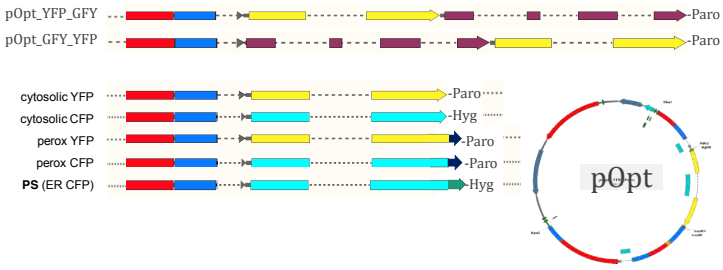
Topology prediction

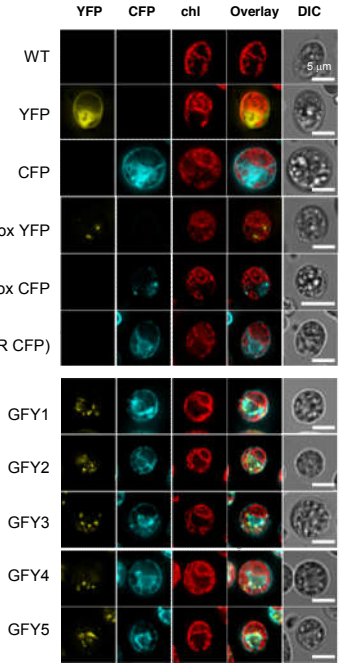


Expression levels

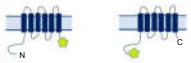


Protein localisation



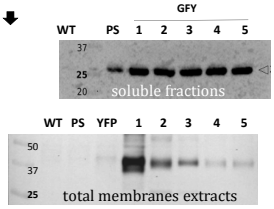


To address protein localisation, GFY genomic sequences were cloned into pOpt backbone vector in both up-/downstream position of YFP variant, mVenus.



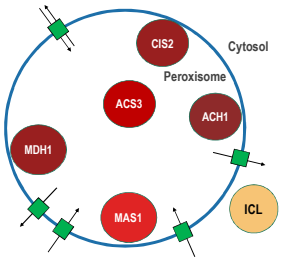
UVM4 parental strain (PS) used for transformation expresses soluble CFP in the ER as an evidence that peroxisomes are ER-derived organelles.

Anti-FP recognizes ~40 kDa proteins localised in total membrane fraction and ~28 kDa protein in the soluble part, demonstrating that chimeric proteins are membrane bound.



Conclusions

Our present data suggest that GFY likely participate to the metabolites exchange between cytosol and peroxisomal microbodies due to the their transporter-like structure.



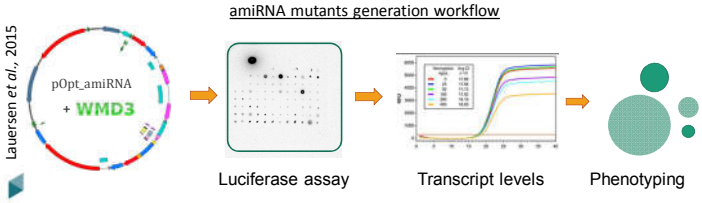
Despite their high similarity in sequence, the differential N-terminal part and expression levels as well, suggest non-identical roles in the carbon metabolism at peroxisomal level.

GFY1 and -2, which are up-regulated in absence of oxygen, could play a not yet understood function regarding metabolites transport in anaerobiosis at the peroxisomal level.

GFY3 and -4 are constitutive expressed both in present or absence of acetate meanwhile GFY5 is strongly up-regulated with acetate especially in the dark. Thus, GFY1-3 could participate to glyoxylate cycle during acetate assimilation pathway and more.

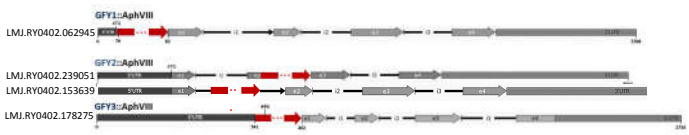
Knock-down GFY4, GFY5 and GFY1-5

amiRNA mutants generation workflow



Knock-out GFY1, GFY2 and GFY3

Chlamydomonas Library Project (CLiP) mutants insertion sites



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