A novel forward genetic screen to identify respiratory complex I mutants in *Chlamydomonas*

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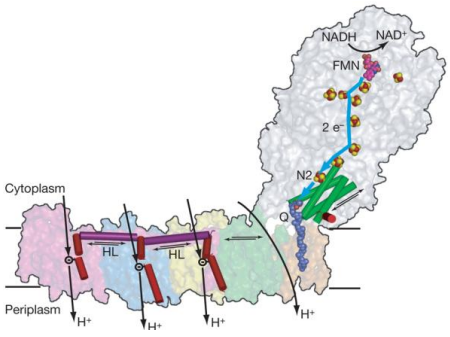
Introduction

NADH:ubiquinone oxidoreductase (complex I) is a ~1000 kDa complex of the mitochondrial respiratory chain. This multiprotein complex transfers electrons from NADH to the ubiquinone pool. The complex I is made of ±45 structural subunits and about a dozen assembly factors have been identified.

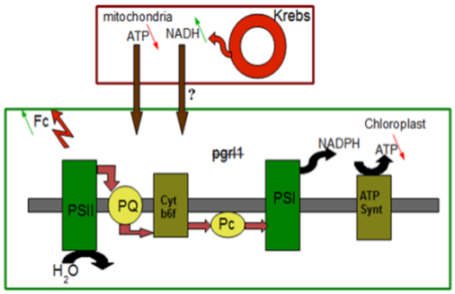
Objectives and summary: Development of a new screening method based on chloroplast/mitochondria interactions in order to unravel new assembly factors of complex I. To achieve such a goal, we first isolated a double mutant, bearing two mutations, one in the *pgrl1* gene leading to inability to generate a correct ATP/NADH ratio in the chloroplast, and one in a subunit of the respiratory complex I. We demonstrated that this *pgrl1/CI-* mutant displays a specific fluorescent phenotype. We are now using the *pgrl1* strain for random insertional mutagenesis using a Hygromycine resistance cassette (HygR) and are looking for clones which possess a similar fluorescence phenotype to our *pgrl1/CI-* double mutant.



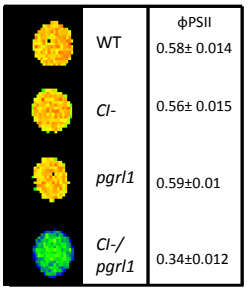
Rouslan G. Efremov et al. The architecture of respiratory complex I.Nature,2010

Screening for mitochondrial mutants using PSII fluorescence

A) Screening principles

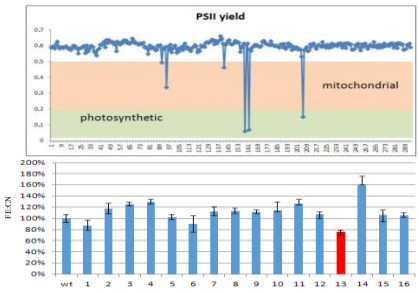


B) Fluorescence analysis



	ϕPSII
WT	0.58± 0.014
CI-	0.56± 0.015
pgrl1	0.59±0.01
CI-/ pgrl1	0.34±0.012

C) Retrieving complex I mutants



A. Modification of the ATP/NADH balance in a double mutant *pgrl1/CI-*. A *pgrl1* strain isn't able to maintain a proper ATP/NADH balance. Normally the lower ATP production is compensated by mitochondria, but the lower ATP production in the *CI-* mutants will disturb this equilibrium, bringing perturbations in the photosynthetic electron chain. The subsequent dysfunction of the photosynthetic chain will appear as a reduced photosynthetic yield. Fc : chlorophyll fluorescence **B.** Comparison of the fluorescence profile directly on plate for different strains (ϕPSII). It is only possible to detect a *CI-* mutation by fluorescence when it is coupled with a *pgrl1* mutation. ϕPSII values for the different strains at 210 µE/m².s.**C. Top.** Fluorescence analysis of 300 transformants obtained from a transformation on a *pgrl1* strain. Any transformant Whose ϕPSII value is between a WT and our reference double mutant is selected for further analysis. The most affected transformants correspond to photosynthetic mutants and can be set aside. **Bottom.** Mutants isolated from the first screening (ϕPSII) are now directly tested for their lack of complex I activity by measuring their Ferricyanide (FE:CN) activity.

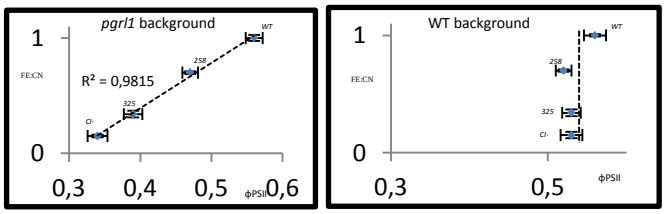
Screening sensitivity

A) FE:CN activity of Complex I mutants

strain	Fe:CN
WT	3256±86
325	1107±95
21	1237±97
258	2236±32
25	690±190

A. FE:CN activity for 4 Complex I mutants isolated among 3000 transformants. FE:CN activity is expressed in nmoles ferricyanide reduced. min^{-1}.mg protéins^{-1}. **B.** relationship between FE:CN activity (standardized to WT activity) and ϕPSII value for complex I mutants differently affected in *pgrl1* background (**left**) and in WT background (**right**)

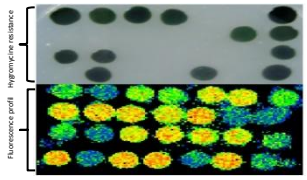
B) FE:CN/ϕPSII relationship



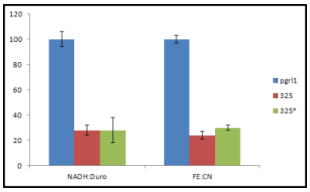
Genetic and molecular analysis

A) Genetic and molecular analysis

Strain	Mutation	Insertion
325	Tagged	Unknown
21	Untagged	Unknown
25	N.A.	ABC Transporter
258	TBD	Kinase



B) Phenotype comparison



A. Left : Results for the cosegregation of the HygR cassette and the *CI-* phenotype and for the localization of the insertion by the TAIL-PCR method. NA : non available; TBD : to be determined. **Right** : Verification of the cosegregation of the *CI-* phenotype and the HygR cassette by fluorescence in the 325 strain. *pgrl1* clones have been retrieved. On such condition, if the mutation is tagged, all HygR clones must have a fluorescence phenotype (green/blue). **B.** Complex I activity on purified membranes for *pgrl1*,325 and 325* (325 with removed *pgrl1* mutation). Activity in nmoles of NADH oxidized. min^{-1}.mg protéins^{-1} (NADH:Duro) and nmoles ferricyanide reduced. min^{-1}.mg protéins^{-1}(Fe:CN).

Conclusion

Using a *pgrl1* strain for random insertional mutagenesis allowed us to identify mitochondrial *CI* mutants. We could identify a variety of differently affected mutants using an quantitative method based on fluorescence values (ϕPSII).

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