Project title: Studying chloroplast biogenesis in the basal land plant *Marchantia polymorpha*: Using CRISPR/Cas9 genome editing and forward genetics to assess functional conservation and seek novel components

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**Project description**

Chloroplasts are responsible for photosynthesis, and are the organelles that define plants [1]. They evolved as a result of an endosymbiotic relationship between a cyanobacterium and an algal progenitor, in a process that began over a billion years ago. Land plants emerged around 500 million years ago, by which time the chloroplast had already become a fully integrated component of the plant cell.

Today, >90% of the ~3000 proteins found inside chloroplasts are encoded by the nuclear genome and synthesized in the cytosol as precursors with N-terminal targeting signals called transit peptides. The import of such precursors into chloroplasts is mediated by multiprotein machines in the chloroplast envelope membranes called TOC and TIC (Translocon at the Outer/Inner envelope membrane of Chloroplasts) [2].

In flowering plants, the TOC complex comprises a channel-forming molecule (Toc75) and multiple receptors in two families (Toc159, Toc34) that recognize precursor proteins as they arrive at the chloroplast surface. Composition of the TOC complex is controlled by a “master regulator” protein called SP1 [3]. The *SP1* gene was identified using a forward-genetic approach in the model flowering plant, *Arabidopsis thaliana*: we screened for extragenic suppressors of a pale-yellow TOC receptor mutant, identifying suppressor mutants by their greener appearance [4]. SP1 is a ubiquitin E3 ligase in the chloroplast outer membrane that targets TOC components for ubiquitination and degradation by the ubiquitin-proteasome system. By controlling protein import in this way, SP1 enables reconfiguration of chloroplast functions in response to developmental and environmental cues [3,4].

Bryophytes, comprising liverworts, mosses and hornworts, are the earliest diverging group of land plants. The liverwort *Marchantia polymorpha* is an emerging model system for plant biology research [5], which because of its basal position in the land plants enables important evolutionary questions to be addressed. *Marchantia* has several distinguishing features, such as the dominance of the haploid gametophyte generation over the diploid sporophyte during its life cycle. The latter point, in combination with its low genetic redundancy, means that *Marchantia* is particularly well suited to forward-genetic screening based on phenotype analysis. Moreover, advanced techniques for generating targeted gene knockouts (including homologous recombination and CRISPR/Cas9 approaches [6,7]) have been successfully applied in *Marchantia*.

We sequenced the *Marchantia* genome [8], and bioinformatic analyses indicated the presence of genes encoding SP1 and all major TOC components. The aims of this project will be to elucidate the functions of these genes, assessing the extent of functional conservation with flowering plants, and to seek entirely new components involved in chloroplast protein biogenesis that eluded detection previously due the higher genetic redundancy in flowering plant models:

1. Reverse genetics. Using CRISPR/Cas9 genome editing [6,7], we will generate knockout or knockdown mutants for *SP1* and all *TOC* genes. The phenotypes of the mutants will then...
be characterized in detail (e.g., in relation to protein import capacity) to elucidate the extent
to which the functions of the genes have been conserved during land plant evolution.
Functional relationships between the components will be assessed by generating and
characterizing all relevant double mutant combinations.

2. Forward genetics. Based on the results from 1, we will establish a forward-genetic
screening strategy to identify entirely novel factors involved in chloroplast protein import. We
predict that TOC mutants will have visible, pale-yellow phenotypes caused by defective
chloroplast biogenesis [2]. This will enable us to conduct a suppressor screen analogous to
that which led to the identification of SP1 in *Arabidopsis* [3]: we will screen for greener plants
following UV mutagenesis. The suppressor mutants will be characterized in detail, and the
mutated genes will be identified by whole-genome sequencing.

References

ligase SP1 is important for stress tolerance in plants. *Curr. Biol.* 25:2527-2534.
5. Ishizaki, K. (2016) Molecular genetic tools and techniques for *Marchantia polymorpha*
7. Sugano, S. et al. (2014) CRISPR/Cas9-mediated targeted mutagenesis in the liverwort
8. Honkanen, S. et al. (2016) The mechanism forming the cell surface of tip-growing rooting

Student profile

This project would suit candidates with a strong background in one or more of the following
areas: biological sciences, molecular biology, cell biology, biochemistry, bioinformatics,
genetics.

Recent publications

Bédard, J., Trösch, R., Wu, F., Ling, Q., Flores-Pérez, Ú., Töpel, M., Nawaz, F. and Jarvis,
P. (2017) Suppressors of the chloroplast protein import mutant *tic40* reveal a genetic link
[Epub ahead of print].

Flores-Pérez, Ú., Bédard, J., Tanabe, N., Lymeropoulos, P., Clarke, A.K. and Jarvis, P.
Physiol.* 170: 147-162.

Ling, Q. and Jarvis, P. (2015) Regulation of chloroplast protein import by the ubiquitin E3
ligase SP1 is important for stress tolerance in plants. *Curr. Biol.* 25:2527-2534.

Trösch, R., Töpel, M., Flores-Pérez, Ú. and Jarvis, P. (2015) Genetic and physical
interaction studies reveal functional similarities between ALBINO3 and ALBINO4 in

Ling, Q. and Jarvis, P. (2013) Dynamic regulation of endosymbiotic organelles by