Artificial microRNA knockdown of fermentative pathways in *Chlamydomonas reinhardtii* and the impact upon H_2 production

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1. Introduction

When cultures of *C. reinhardtii* are deprived of sulphur, the rate of photosynthesis drops below respiration triggering anaerobic H_2 production. Electrons are fed to the hydrogenase from residual PSII activity and the fermentative breakdown of starch reserves [1]. We aim to investigate fermentative pathways believed to compete with H_2 production [2] (Figure 1) by artificial microRNA (amiRNA) knockdown [3], with a view to increasing in H_2 yields. We describe here the construction and characterisation of a knock-down mutant of pyruvate formate lyase (PFL1) which is predicted to be involved in the anaerobic production of formate.

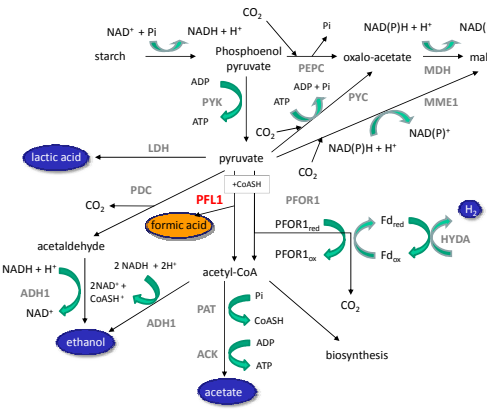

Figure 1: Schematic of proposed fermentative pathways in *C. reinhardtii*, acetate kinase (ACK), bifunctional acetylaldehyde/alcohol dehydrogenase (ADH), ferredoxin (Fd), fumarate reductase (FMR), fumarase (FUM), [Fe]-hydrogenase (HYDA), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), malic enzyme (MME1), phosphate acetyl transferase (PAT), pyruvate decarboxylase (PDC), phosphoenol pyruvate carboxylase (PEPC), pyruvate formate lyase (PFL1), pyruvate ferredoxin oxidoreductase (PFOR), pyruvate carboxylase (PYC), pyruvate kinase (PYK). Adapted from [2].

2. Creating amiRNA knockdown lines in *C. reinhardtii*

•Knockdown mutants were created using vector pChlamiRNAi3 (Figure 2A) to drive expression of artificial pre-miRNAs designed with online tool *Web MicroRNA Designer* (WMD3).

•Transformants screened by immunoblot identified knockdowns with 70-80% reduction in the levels of PFL1 compared to wild type (wt) CC-406 (Figure 2B).

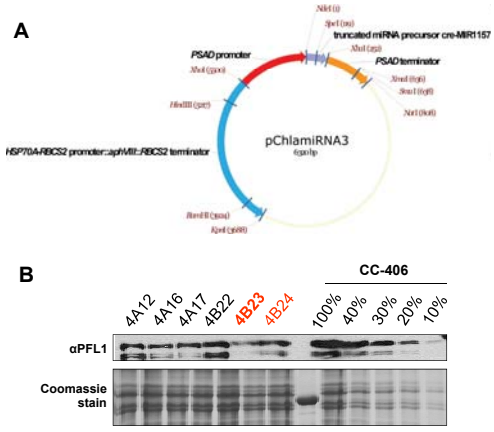

Figure 2A Map of amiRNAi vector pChlamiRNA3 (http://www.plantsci.cam.ac.uk/research/baulcombe/sequencedata.html) used to transform *C. reinhardtii* wild type strain CC-406. aphVIII resistance cassette allows for selection on paromomycin and PsaD promoter used to drive expression of amiRNAs designed using online tool WMD3 (http://wmd3.weigelworld.org/cgi-bin/webapp.cgi) **Figure 2B** Immunoblot screening of transformants for knockdown of target enzyme PFL1, loading is shown by Coomassie stained SDS PAGE gel.

3. Effect of growth conditions on knockdown expression

•amiRNA knockdown under the control of the PsaD promoter was increased by photoautotrophic conditions (Figure 3A, 3B).

•Knockdown of PFL1 in *C. reinhardtii* had little effect on growth.

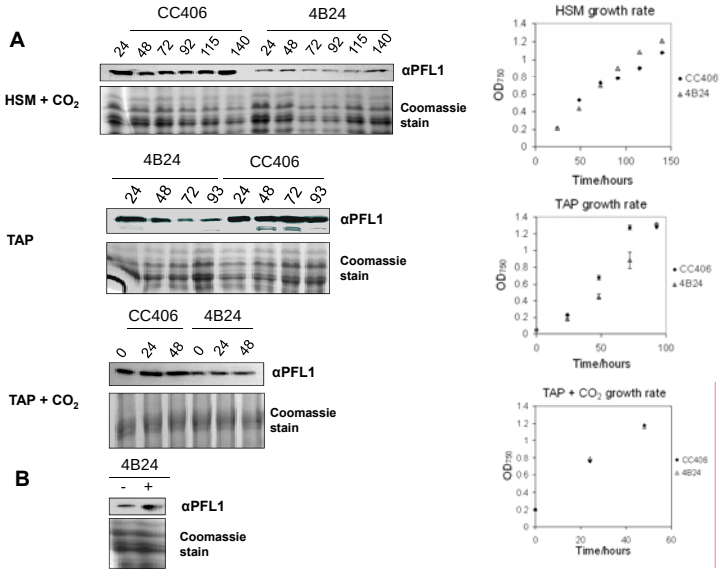

Figure 3A Immunoblot analysis of PFL1 levels in knockdown mutant 4B24 as function of growth under photoautotrophic, mixotrophic and heterotrophic conditions, loading given by Coomassie stained SDS PAGE gel. **Figure 3B** Effect of acetate on amiRNAi knockdown of PFL1, (OD_{750}=0.847) HSM CO_2 grown culture (-) re-suspended in TAP and bubbled with CO_2 for 2 hours (+).

4. Effect of PFL1 knockdown on H_2 production under sulphur deprivation (TAP-S)

•In contrast to wt CC406, PFL1 was not induced in knockdown strain 4B24 (Figure 4A) during sulphur deprivation.

•4B24 showed increased gas evolution compared to wt CC406 (Figure 4B).

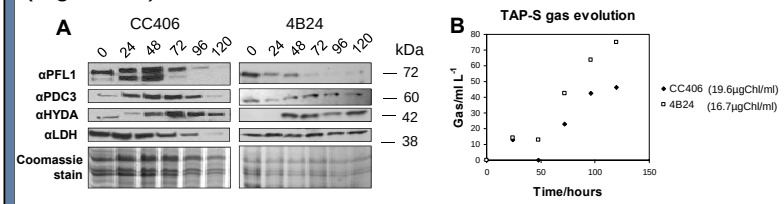

Figure 4A Immunoblot analysis of fermentative enzyme expression in wt (CC406) and PFL1 knockdown (4B24) strains after sulphur deprivation, loading given by Coomassie stained SDS PAGE gel. 325ml cultures were sealed, incubated at 25ºC and 140µEm^{-2}s^{-1} continuous illumination. **Figure 4B**. Gas evolution of sulphur deprived cultures measured by water displacement

5. Conclusions

•The use of artificial microRNAs for targeted gene knockdown opens up exciting new opportunities for reverse genetics in *Chlamydomonas reinhardtii*.

• Switching off fermentative pathways is a promising means of increasing hydrogen production as demonstrated in the PFL1 knockdown mutant 4B24.

REFERENCES

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