IS3.5 - NDH-1 Complexes in Cyanobacteria: How are they coupled to CO_2-uptake? Are they reversible?

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The cyanobacterial high affinity **CO_2-concentrating mechanism** (**CCM**) efficiently supplies CO_2 to the photosynthetic mechanism of cyanobacteria. Essentially, it functions as a 'supercharger' for CO_2 by concentrating it within the cytoplasm of the cell, thereby saturating the active sites of the CO_2-fixation enzymes and thus increasing the efficiency of photosynthesis.

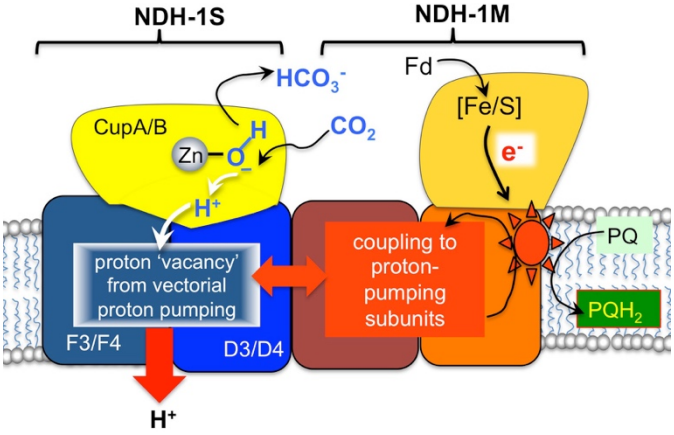

Figure 1. Hypothesis for electron transfer coupled to proton pumping coupled to CO_2-hydration. Hypothetical metal center, possibly Zn^{2+} ion. CupA/B may provide all or some of the ligands (likely His and/or Cys) to the metal center. The metal facilitates the de-protonation of substrate H_2O forming a hydroxide capable of nucleophilic attack upon incoming CO_2 as in the case of carbonic anhydrases. Proton pumping activity removes H^+ from the active site to trap the deprotonated metal hydroxide formed upon deprotonation of the metal-bound H_2O.

To begin testing this hypothesis, we constructed a double knockout mutant, where no NDH-$1_{3,4}$ is produced and introduced the whole *cupA* operon under Rubisco promoter control and/or *cupB* under *rnpB* promoter in an ectopic genomic site. Physiological analysis of C_i depleted mutants with chlorophyll fluorescence traces and O_2 evolution dependent on C_i, show a high C_i requirement feature on the double knockout mutant. However, the strain expressing constitutively NDH-1_3 have lost its high CO_2-requiring phenotype, displaying restored cell CO_2 uptake. Initial studies analyzing the effect of point mutations on conserved His/Cys of CupA/CupB protein to evaluate their potential role on CO_2 uptake in cyanobacteria will be presented.

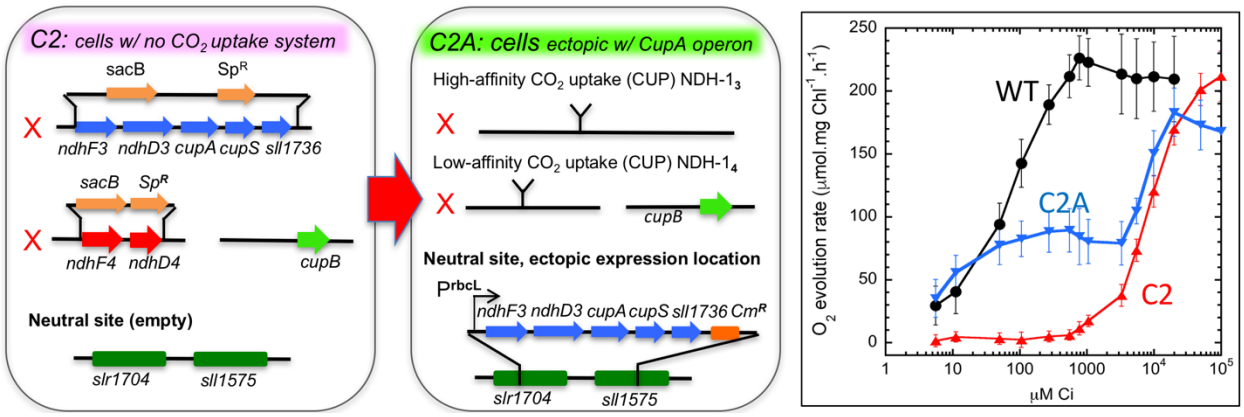

Figure 2. Cup-less strain, C2 and transformant C2A (Left Panels). The marker-less strain C2 lacks the gene regions for NDH-$1_{3,4}$ was the recipient of the ectopic construct where CUP operon (NDH-1_3) is under the control of a rubisco promoter integrated on a neutral site (C2 + CupA operon →C2A). **The C2A strain has a constitutive expression the high affinity, low flux NDH-1_3 system** (blue) compared to the full NDH-1_3 plus NDH-1_4 knockout (red), yet cells are incapable of both high affinity and high flux uptake observed in the wild-type (black) (**Right Panel**)