Towards a global understanding of the nitrogen-stress responses of *Synechococcus elongatus*/ *Synechocystis* PCC6803

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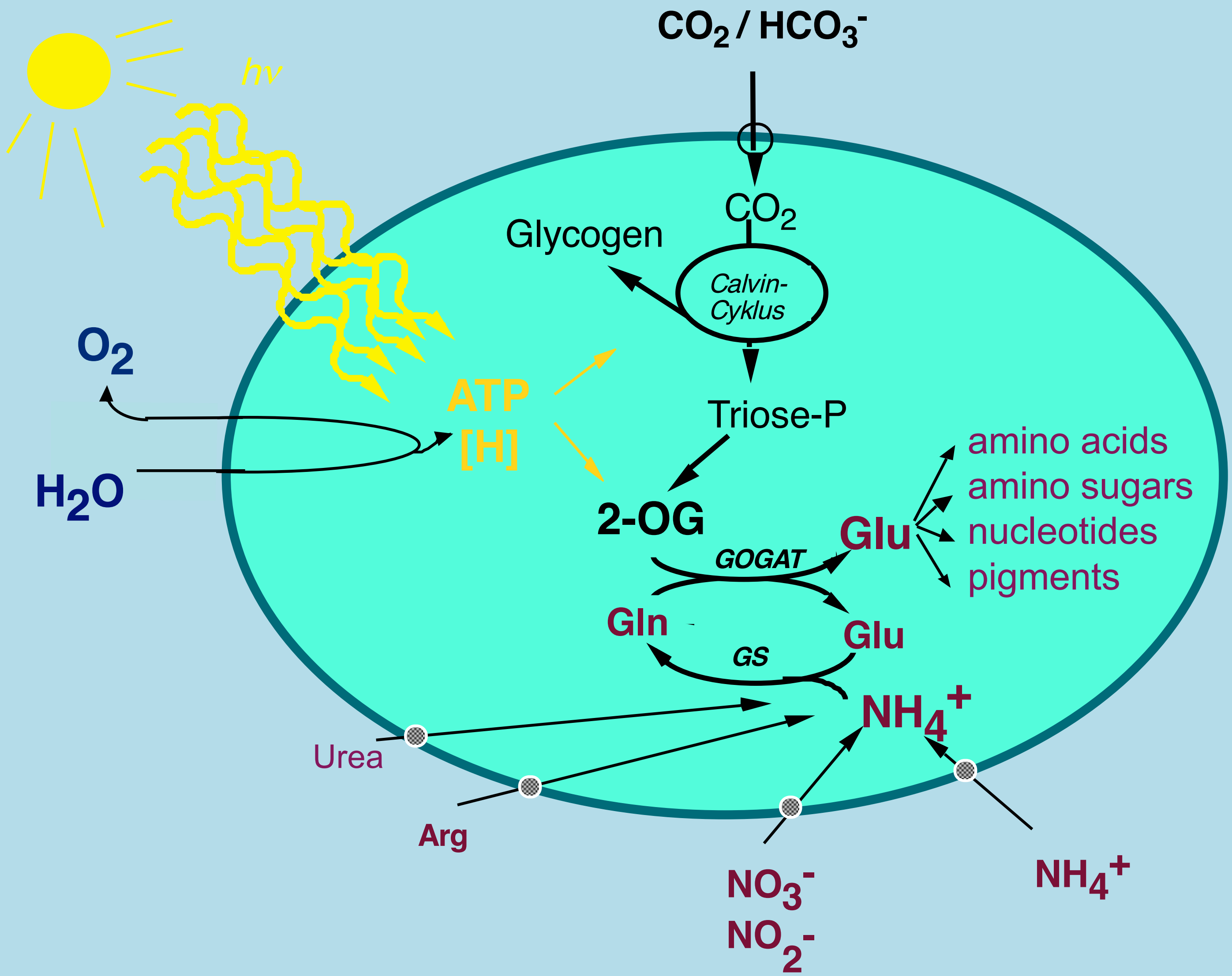
The nitrogen-stress responses of non-diazotrophic cyanobacteria:

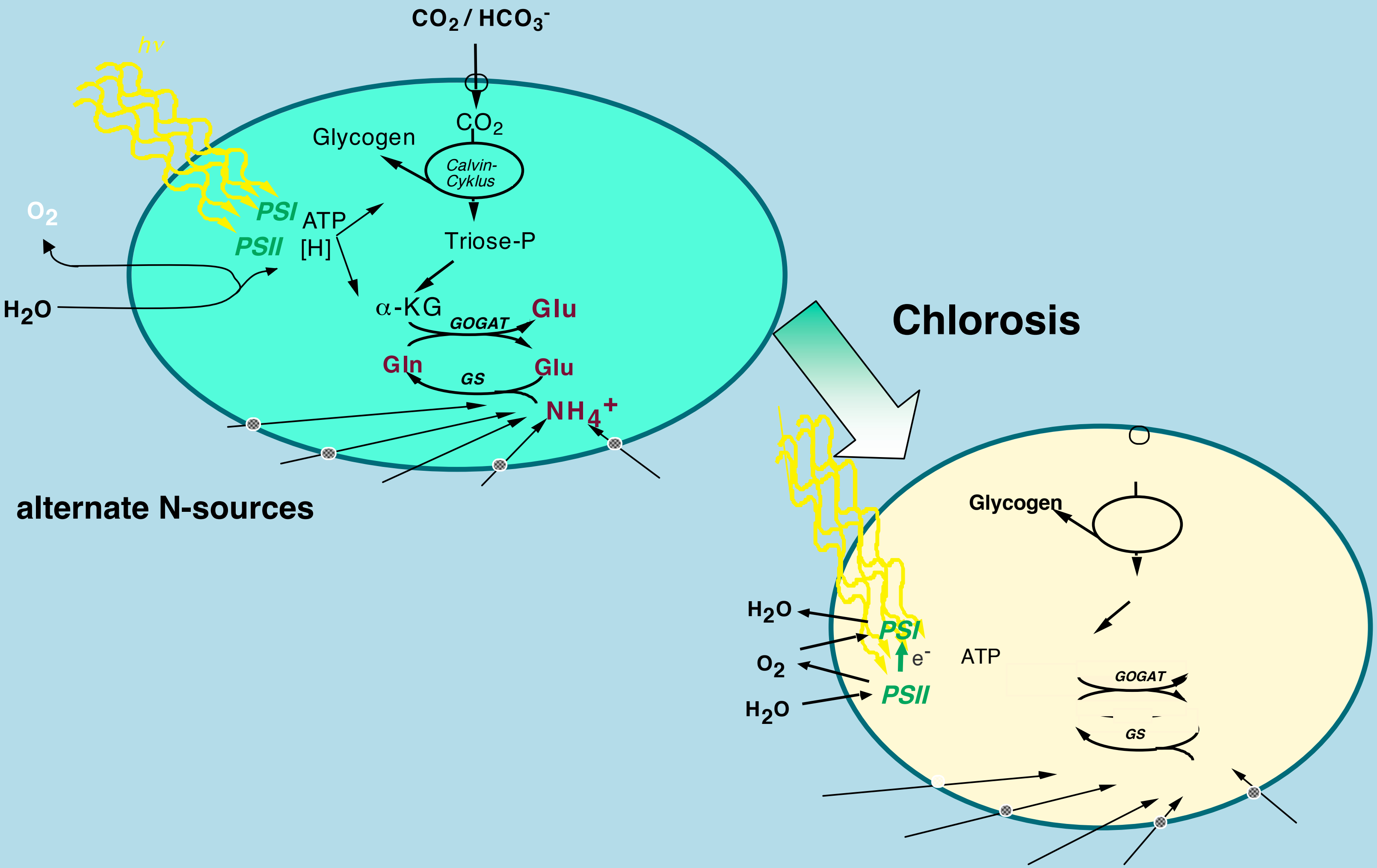
No stress: Nitrogen sources are present at optimal concentrations



Nitrogen starvation stress:

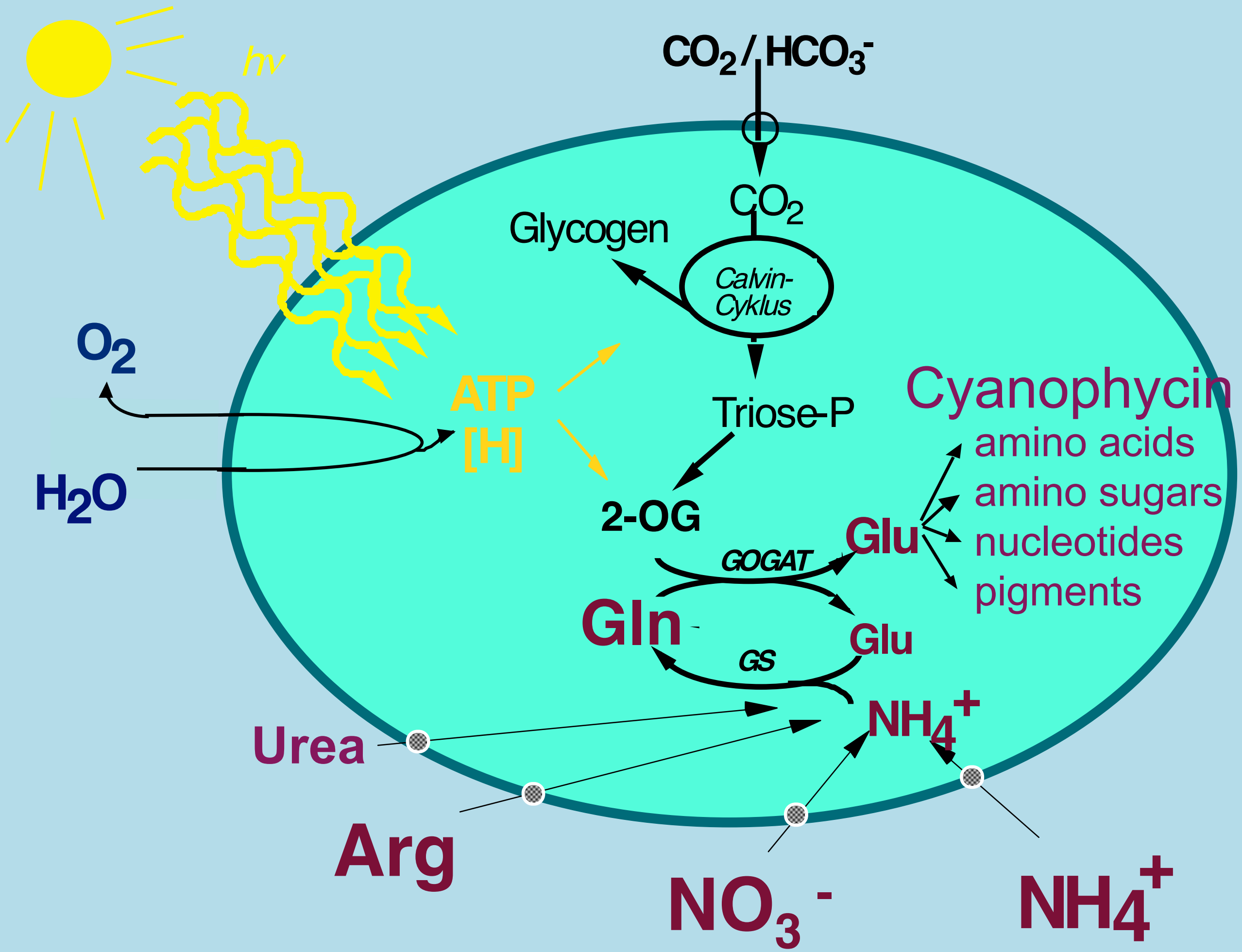
1. Cells try to acquire alternate N-sources

2. Acclima(ta)tion to a non-growing, dormant-like state (chlorosis)



Nitrogen Excess Stress:

1. Cells avoid assimilation of excessive ammonia: may compromise the Glutamate pool
2. Cells avoid the synthesis of surplus of ammonia: too much is toxic
3. If external ammonia is in excess: increase ammonia tolerance

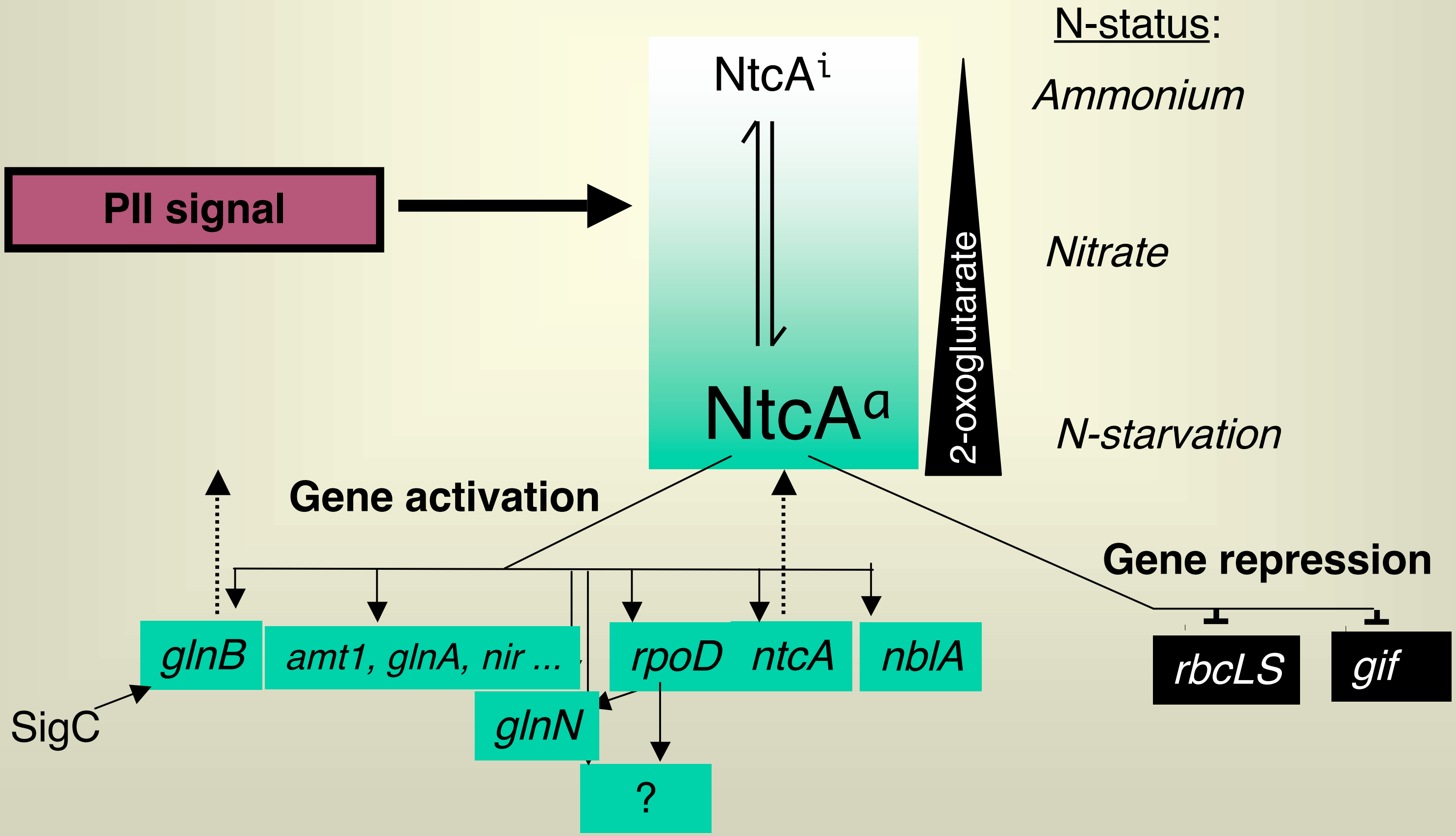


Sensing the Nitrogen status: perception of the cellular 2-oxoglutarate state

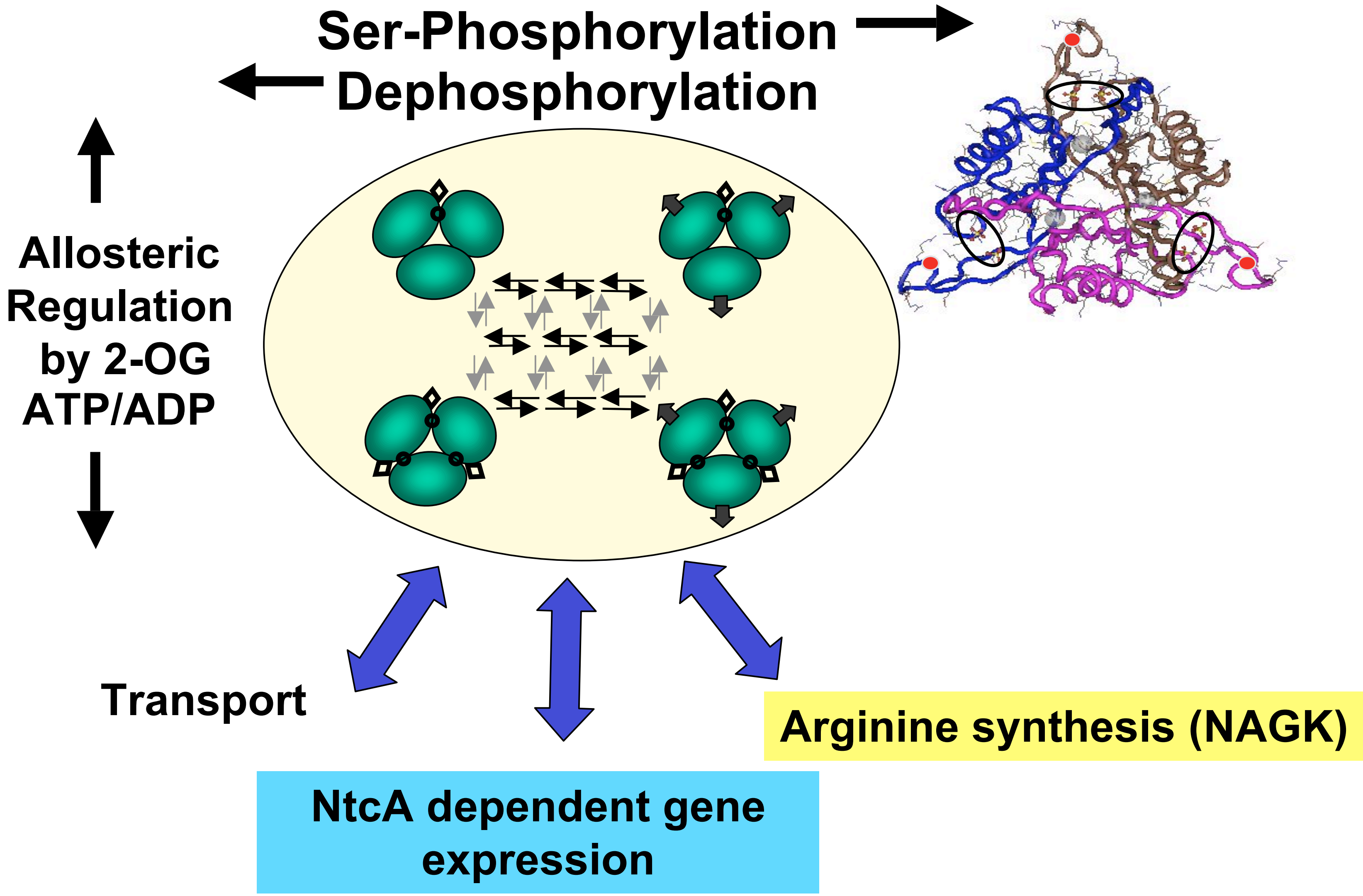
2-OG sensors: NtcA and PII:

mediate nitrogen responses at the transcriptional and port-transcriptional level

NtcA: a global transcription factor, which, during nitrogen-limiting conditions, enhances the expression of genes involved in nitorgen assimilation and represses several other metabolic genes.

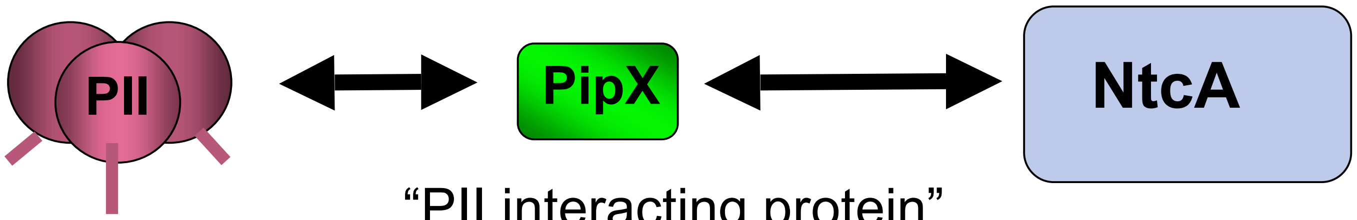


PII Signaling in Cyanobacteria



PII control over NtcA : mediated by PipX

Yeast-Two Hybrid Screening:



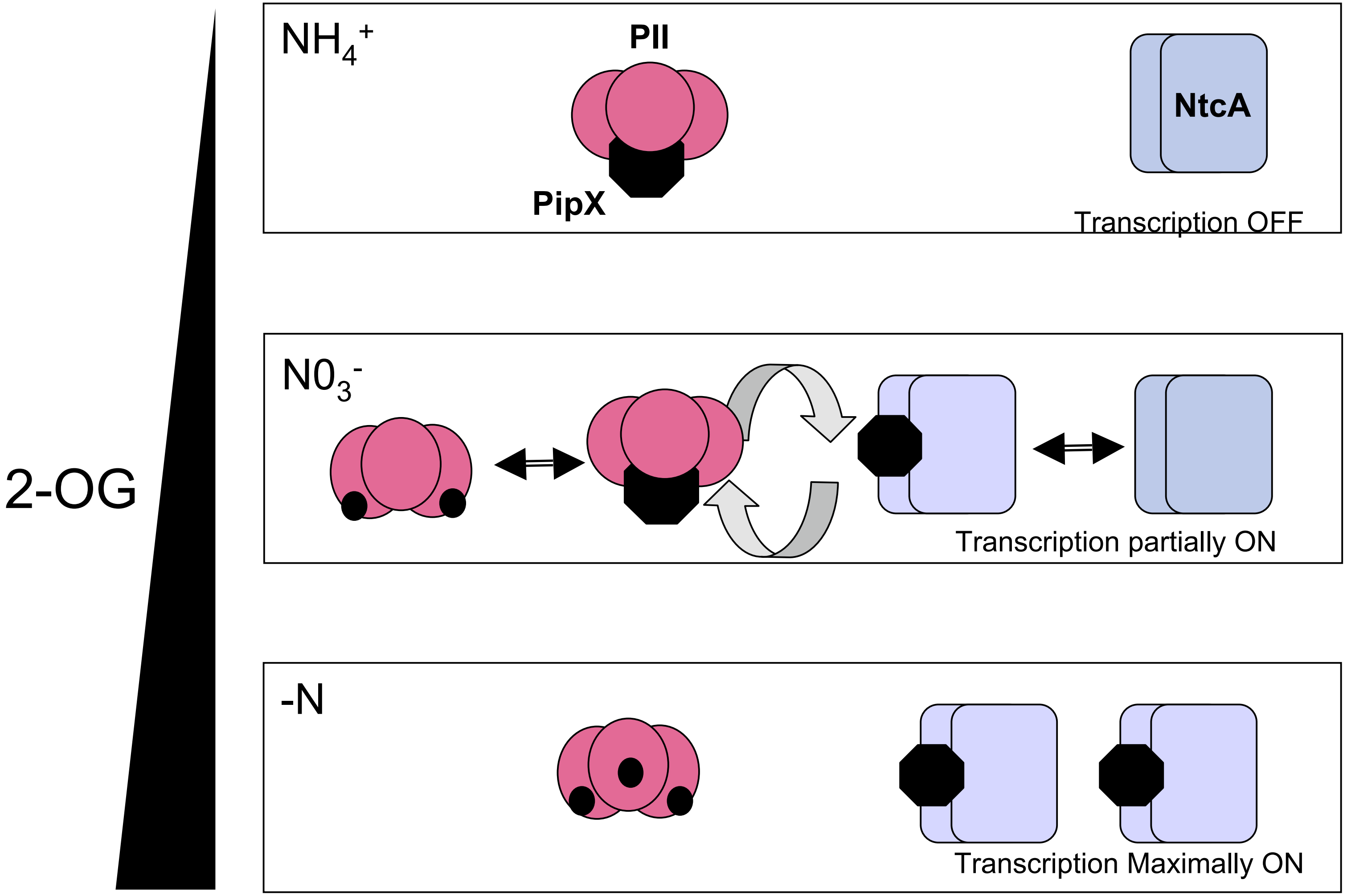
“PII interacting protein”

Biochemistry:

Interaction impaired by ATP + 2-oxoglutarate
Insensitive towards PII-Phosphorylation

Interaction stimulated by 2-oxoglutarate

PipX is a transcriptional co-activator of NtcA



Espinosa et al., *Mol. Microbiol.* **61:** 457-466

General starvation stress responses during nitrogen deprivation: the chlorosis response

The enigmatic regulator NblR in *Synechococcus elongatus*

Homologue to bacterial respose regulators of 2-component systems (OmpR/PhoB family)

NblR deficient mutant: non-bleaching phenotype during high-light, nitrogen and sulfur-starvation due to impaired phycobiliprotein proteolysis

NblR mutant fails to enhance expression of the *nblA* gene, which is required for induction of phycobiliprotein degradation.

Expression of *nblA* also requires the NtcA transcription factor during N-starvation.

What is the contribution of NtcA-dependent and NblR-dependent regulation during transition to nitrogen deprivation conditions?

Synechococcus elongatus PCC7942 Microarray: Genom-Zentrum Bielefeld (Anke Becker)

Comparative microarray analysis of *Synechococcus elongatus* wild-type and four mutants: NtcA- PipX- PII- and NblR-deficient

Cells shifted from BG11-Ammonium to -N conditions

143 gens induced (m > 0.89) **in Wt by N-starvation: of those,**

55 dependent on NtcA, 38 on NblR (of those, 35 in common)

103 genes repressed (m < -0.89) **in Wt by N-stravation: of those,**

6 dependent on NtcA, 7 on NblR (2 in common)

Most of the NtcA-upregulated genes fall in two functional categories:

1. **Unknown function**
2. **Related to nitrogen metabolism**

- A large fraction of the genes induced upon N-starvation belongs to the NtcA regulon, but only a few of the repressed genes require NtcA

- The co-activator PipX is always required for full induction, but not for repression

- In the NblR mutant, full expression of many NtcA-dependent genes is impaired

- No NblR-specific genes could be identified

NblR appears to be a modulator of gene expression under conditions of starvation (Nitrate vs. Ammonium response is not impaired in NblR-mutant)

Ammonium acclimation

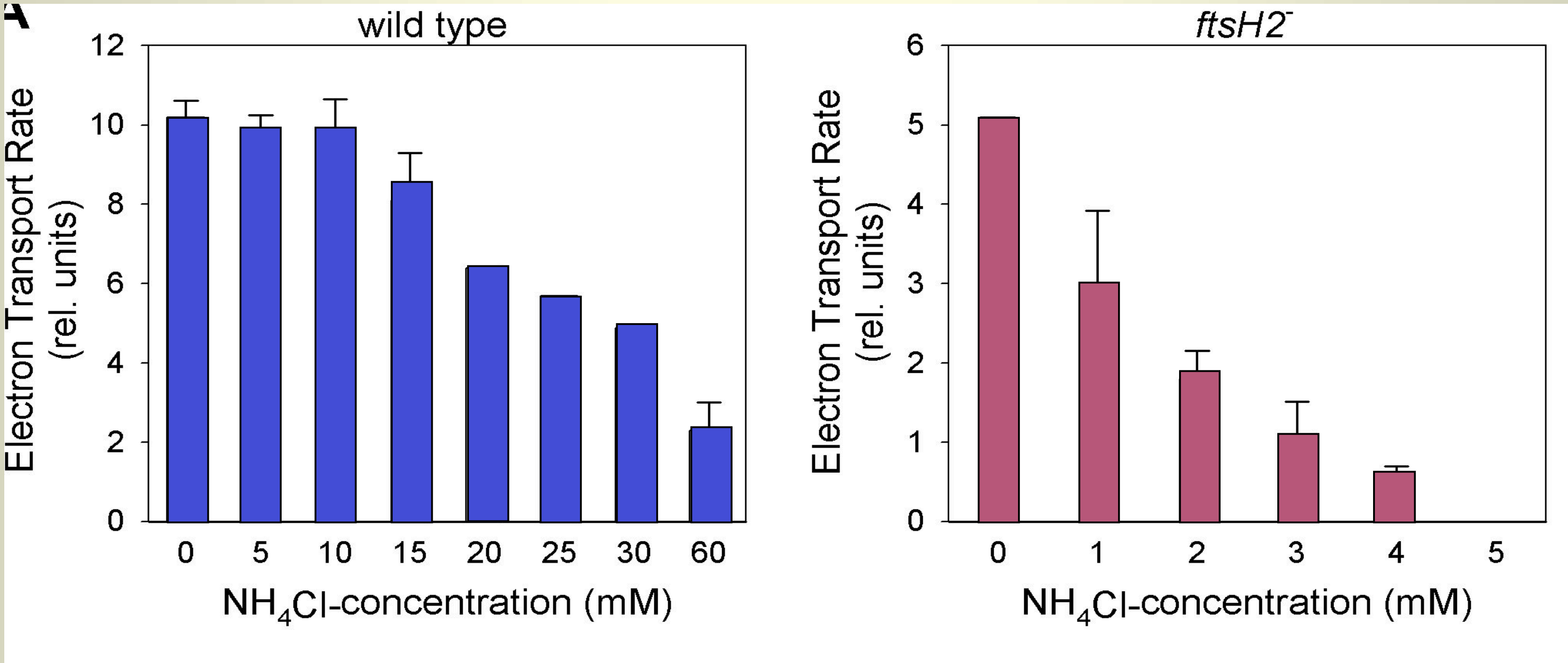
First line of defense:

- **Inhibition of amt activity,**
- **Inhibition of GS activity (to avoid depletion of the Glu pool)**
- **Inhibition of utilization of alternate N-sources (PII and NtcA)**
- **Repression of the NtcA regulon**

Ammonium acclimation

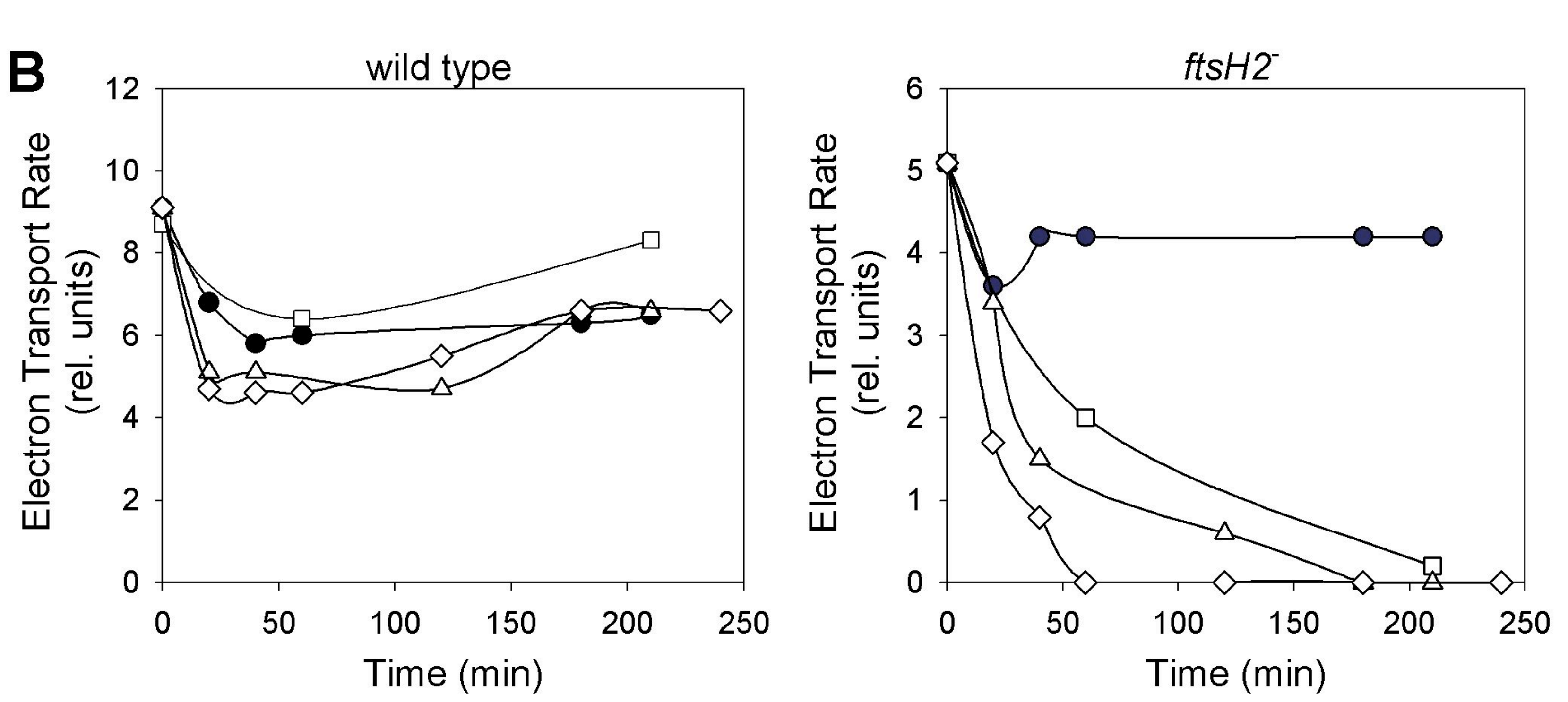
Second line of defense: enhance tolerance towards ammonium

The protease FtsH2 in *Synechocystis* PCC 6803 confers ammonium tolerance

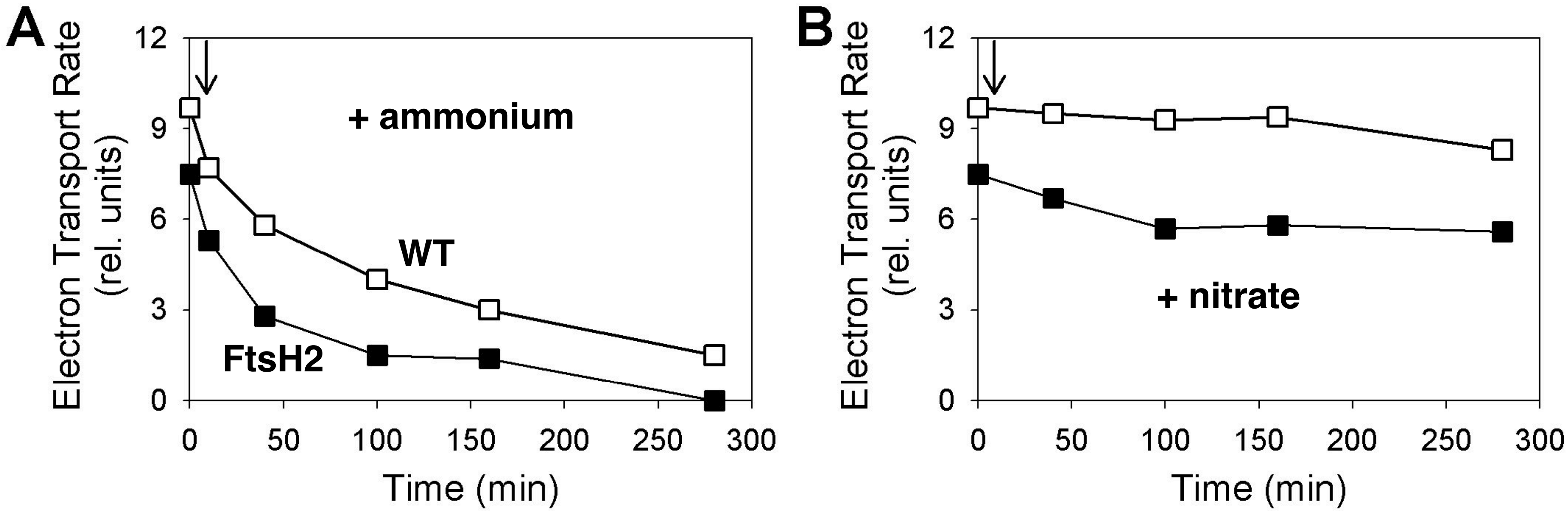


FtsH2: a key player in the PSII repair cycle

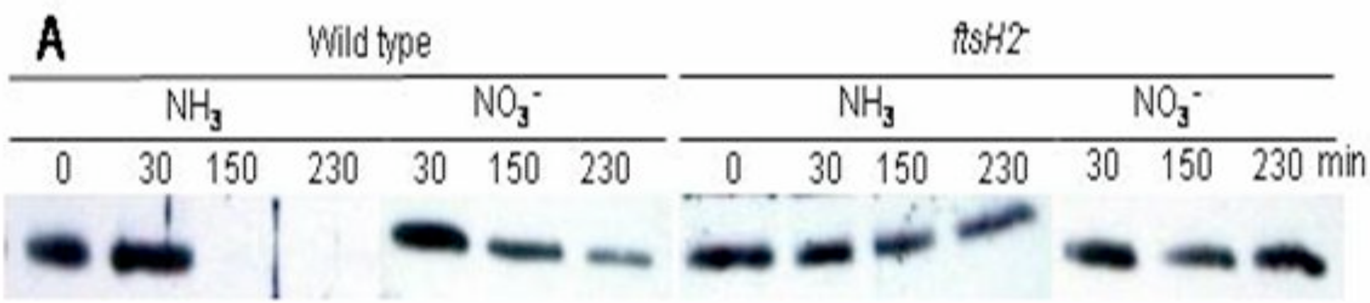
Ammonium toxocity is light dependent



Protein synthesis (PSII repair) is required for ammonium tolerance in of wild-type cells



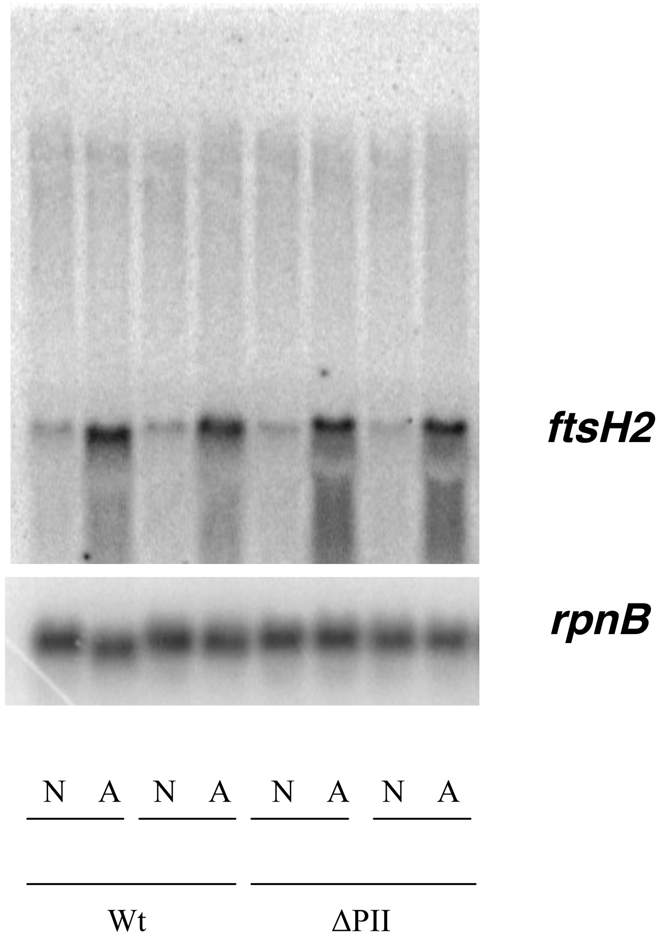
D1 Protein is rapidly destroyed in the presence of ammonium



--> **Ammonium toxicity is due to enhanced photodamage of PSII**

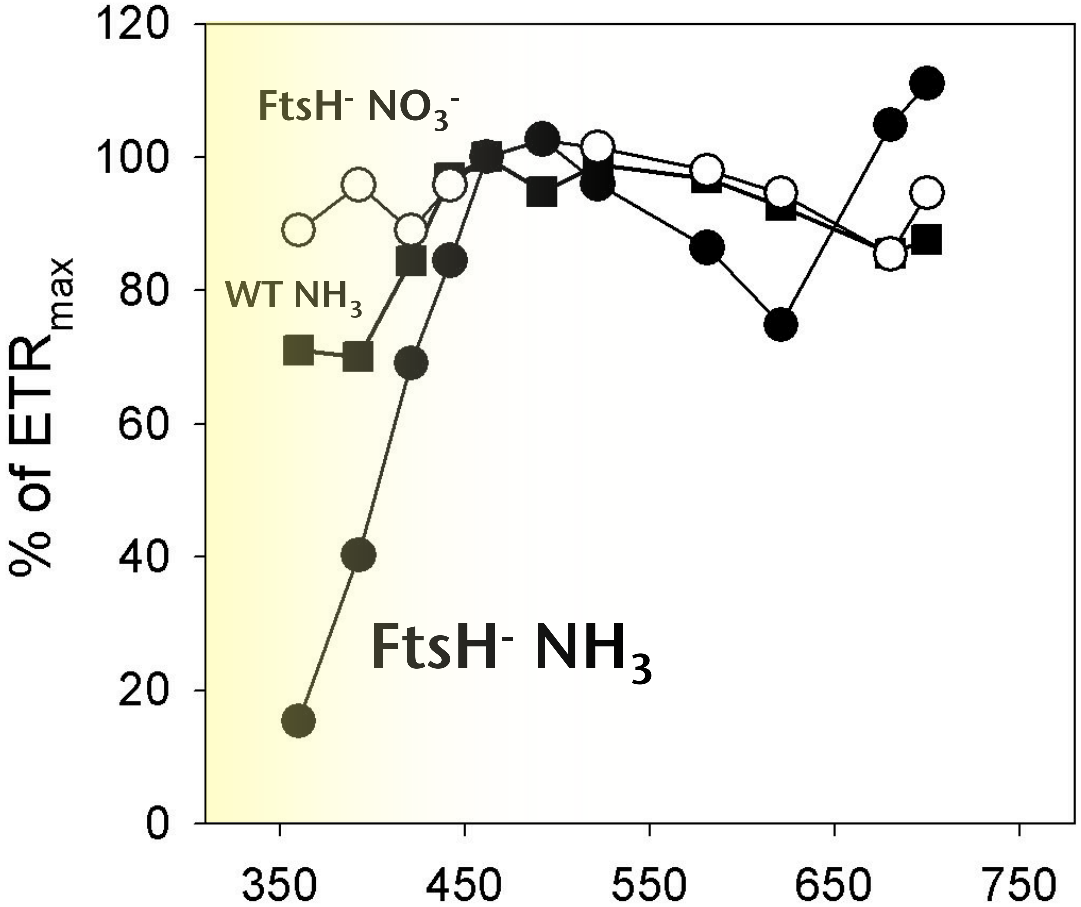
Ammonium tolerance requires an efficient PSII repair cycle

Expression of *ftsH2* is induced by ammoium in presence of light:

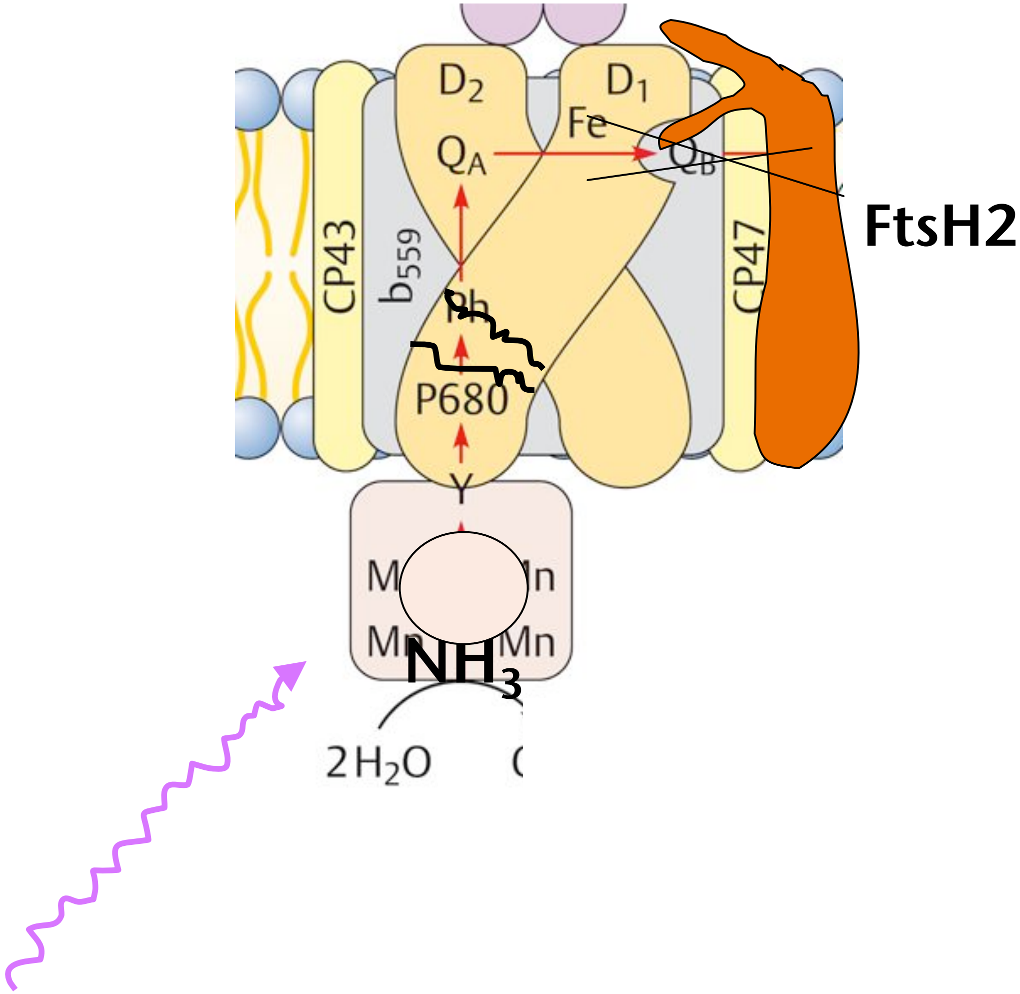


How does ammonia damage PSII?

Action spectrum of ammonium/photodestructive light



Light absorbed by the Mn-Cluster


D2
D1
Fe
QA
QB
FtsH2
CP43
b559
CP47
P680
Y
Mn
NH3
2H2O

Thanks to Co-Workers and Partners

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