# On the role of N-terminal D1 phosphorylation in the PSII repair cycle of higher plants

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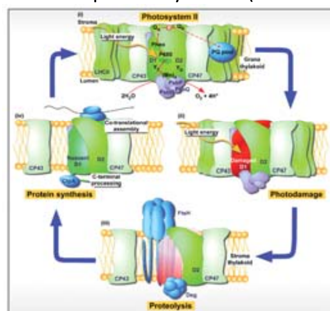
## Importance of phosphorylation for PSII repair

PSII, which catalyses the oxidation of water to oxygen in photosynthesis, is constantly damaged by light. Its repair is highly coordinated and is thought to be dependent on the phosphorylation state of PSII core proteins catalysed by kinases such as STN7 and more specifically STN8 (Tikkanen and Aro, 2011).

The PSII repair cycle mainly replaces damaged D1, which is the main target of photodamage. However, the use of kinase mutants only indicate a global pattern of dephosphorylation and a precise assay for each PSII core protein examination appears to be required.



PSII repair cycle in higher plants, taken from Kato and Sakamoto (2009). Damaged D1 (red) is removed, degraded and then replaced by a new copy.

## Strategy for D1 protein phosphorylation study

To investigate the role of individual phosphorylation sites, a mutagenesis strategy based on tobacco chloroplast transformation has been devised. Our focus was on the Threonine residue at position 2 (T2) of the D1 protein, which is the mainly targeted subunit of the PSII repair cycle. Point mutations, as well as a silent NdeI site, were introduced in several transformation vectors, and the selectable marker was inserted as close as possible to the mutations.

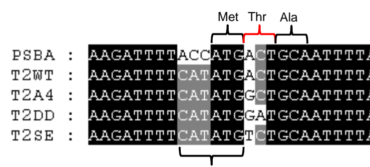
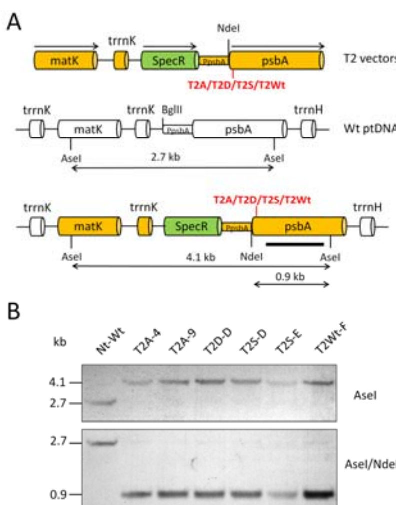



Figure 2. DNA sequence alignment of the region corresponding to the N-terminus of D1 in vectors pFMT2Wt, pFMT2A, pFMT2D, pFMT2S and wildtype psbA gene.

## Generation of homoplastomic T2 mutants in tobacco

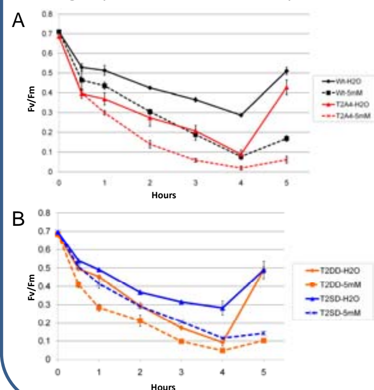
Several independent T2 mutant lines were obtained by biolistic bombardment and growth on selective media. After identification of positive transformants by PCR and several rounds of selection, a Southern blot analysis revealed that all T2 mutant lines were homoplastomic, as indicated by the 4.1 and 0.9 kb fragments obtained after digestion by AseI and AseI/NdeI, respectively. The N-terminus of D1 was sequenced to ensure the mutation was present in all phototrophic lines.



Generation of homoplastomic T2 mutants lines. (A) Schematic representation of the homologous recombination during chloroplast transformation between wildtype DNA (ptDNA) and T2 vectors (T2 Vectors). (B) Southern blot analysis of total genomic DNA extracted from T2 mutant lines using restriction enzymes AseI alone or together with NdeI. The detection was performed using a DIG-labelled probed made by amplifying the *psbA* gene.

## Effect of T2 mutations on the PSII repair cycle

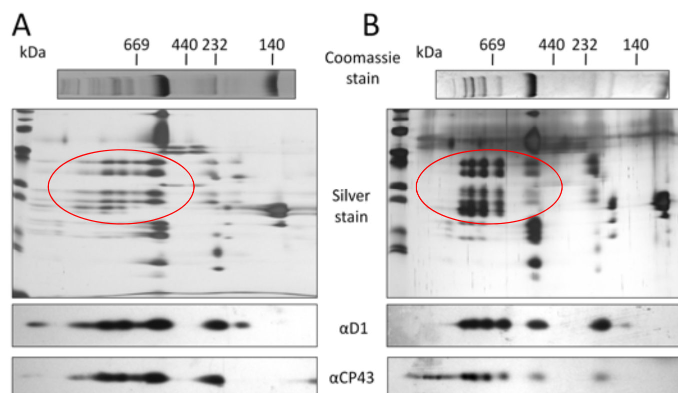
Residue exchange mutations at position T2 had **NO** influence on the transplastomic plants under normal growth conditions with respect to D1 protein levels or PSII and PSI fluorescence characteristics. However, during high-light treatment, T2Wt and T2S had wild-type PSII repair activities, while T2A and T2D suffered stronger photoinhibition, despite a functional PSII repair cycle.



T2 mutant lines (A, Wt and T2A4; B, T2DD and T2SD) grown at about 100 µE*m$^{-2}$*s$^{-1}$ were dark adapted overnight, then exposed for 3 h to 10 µE*m$^{-2}$*s$^{-1}$ in the presence of water ($H_2O$) or 5 mM lincomycin (5 mM). They were then subjected to about 800 µE*m$^{-2}$*s$^{-1}$ for 4 h. After that, they were transferred back to 10 µE*m$^{-2}$*s$^{-1}$ overnight (5). Fv/Fm values were taken at 0, 0.5, 1, 2, 3 and 4 h and after overnight incubation (5). Values represent triplicates.

## Impact of perturbed N-terminal D1 phosphorylation at position T2 on PSII-LHC complexes

The influence of the lack of regulation by phoshorylation at the N-terminus of the D1 protein was assessed by 2D Blue Native (BN)/SDS PAGE followed by immunoblotting with specific antibodies (αD1 and αCP43) of thylakoid membranes isolated from *N. tabacum* Wt (A) and the T2A4 (B) line. The results indicate that the proportion of large PSII-LHC complexes is increased in the T2A4 line compared to Wt.



β-Dodecylmaltoside solubilised thylakoid membranes isolated from *N. Tabacum* wildtype (A) and the T2A4 mutant line (B) were separated on a BN-PAGE (4-12% (w/v)) and Coomassie stained. The lanes were then cut, incubated in solubilisation buffer and used for a 2nd dimension run on a 15 % (w/v) SDS PAGE gel, before silver staining. Immunoblots of 2nd dimension gels were probed with antibodies specific for D1 and CP43.

## Conclusions

**1-** A lack of phosphorylation of the D1 protein at residue T2 does not affect growth of tobacco under normal laboratory growth conditions.

**2-** N-terminal phosphorylation of D1 is not necessary for PSII repair under high-light conditions in higher plants.

**3-** The lack of regulation of D1 phosphorylation may be important for the correct assembly of PSII-LHC complexes to prevent photoinhibition.

**4-** The reduced levels of CP43 in the T2A mutant may be a result of its proximity and interaction with the N-terminus of the D1 protein.

## References

- Tikkanen and Aro (2011) *Biochim Biophys Acta.* Epub ahead of print May 14
- Kato and Sakamoto (2009) *J Biochem.* **146**:463-469.

## Acknowledgements

FM, MB and PJN were supported in this work by the BBSRC and EPSRC.