Data Article

Data supporting the absence of FNR dynamic photosynthetic membrane recruitment in trol mutants

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In photosynthesis, the flavoenzyme ferredoxin:NADP+ oxidoreductase (FNR) catalyses the final electron transfer from ferredoxin to NADP+, which is considered as the main pathway of high-energy electron partitioning in chloroplasts (DOI: http://www.dx.doi.org/10.1111/j.1365-313X.2009.03999.x [1], DOI: http://www.dx.doi.org/10.1038/srep10085 [2]). Different detergents and pH treatments of photosynthetic membranes isolated from the Arabidopsis wild-type (WT) and the loss-of-function mutants of the thylakoid rhodanase-like protein TROL (trol), pre-acclimated to either dark, growth-light, or high-light conditions, were used to probe the strength of FNR-membrane associations. Detergents β-DM (decyl-β-D-maltopyranoside) or β-DDM (n-dodecyl-β-D-maltopyranoside) were used to test the stability of FNR binding to the thylakoid membranes, and to assess different membrane domains containing FNR. Further, the extraction conditions mimicked pH status of chloroplast stroma during changing light regimes. Plants without TROL are incapable of the dynamic FNR recruitment to the photosynthetic membranes.

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### Value of the data

- Data assess alternative membrane binding and release of chloroplast FNR, or its association with different membrane complexes.
- Recruitment of FNR to thylakoids was tested on WT or trol Arabidopsis plants pre-acclimated to different light conditions.
- Biological function of FNR-membrane association in the context of photosynthetic electron flow regulation was addressed.

### 1. Data

The data indicate that the absence of TROL protein influences the dynamic membrane association properties of chloroplast FNR. Thylakoids were isolated from plants acclimated to different light regimes by using buffers of different pH and containing either nonionic detergents decyl-β-D-maltopyranoside (β-DM) (Fig. 1b), or n-dodecyl-β-D-maltopyranoside (β-DDM) (Fig. 1c), or no detergent (Fig. 1a). The inclusion of β-DM or β-DDM was used to probe the stability of FNR binding to thylakoid supramolecular complexes and to assess different membrane domains containing FNR. The dynamism of TROL-FNR interaction was evaluated by quantifying FNR distribution between the membrane and the soluble fractions.

### 2. Experimental design, materials and methods

#### 2.1. Plant material and growth conditions

*Arabidopsis thaliana* (L.) ecotype Columbia (Col-0) plants and *At4g01050* knock-out mutant line, *trol* [1], were grown on potting substrate (Stender, Germany) in the growth chamber (Kambič, Slovenia).
Fig. 1. The distribution of FNR in the thylakoids of Arabidopsis WT and the trol plants grown under different light regimes and isolated under different pH conditions. Thylakoids isolated from Arabidopsis WT and trol plants were treated by different pH, in the absence of the detergent (a), or in the presence of β-DM (b), or β-DDM (c). Subsequently, membrane and soluble fractions were separated by ultracentrifugation into pellet (P) and supernatant (S), analyzed by using SDS-PAGE, followed by Western transfer and immunodecoration with the α-FNR. The α-AtpB and the α-Lhca2 were used as loading and extraction controls. ECL was used for final detection. Graphs represent the percentages of insoluble (P, black columns) and soluble fractions (S, gray columns), after the treatments. Trend lines were drawn according to the mean values of P/S for each data set to emphasize the differences in FNR distribution. The slope of the line (m) was calculated and indicated for each trend line. Graphs depict the representative data of the three independent experiments.
Conditions were: 20°C, 80 μmol photons m⁻² s⁻¹ (Osram Flora, Osram, Germany) with a 16-h light photoperiod and a relative air humidity of 60% (day), or 70% humidity (night). Prior to analyses, plants were pre-acclimated for two days either to dark, or to 80 μmol photons m⁻² s⁻¹ (GL), or to 250 μmol photons m⁻² s⁻¹ (HL) (Envirolite fluorescent lamps, Envirolite, Great Britain). The trol line has been characterized in detail previously [1].

2.2. Chloroplast isolation

Intact Arabidopsis chloroplasts were isolated from 3- to 4-week old plants as described by Vojta et al. [2]. Final sedimentation was achieved by centrifugation at 1,000 g for 5 min. Sediment was resuspended in the buffer containing 330 mM Sorbitol and 20 mM Tris/HCl pH 8.4. Chloroplast concentration was set to 1 mg chlorophyll per 1 ml buffer. Chloroplast intactness and functionality was assessed by oxygen evolution measurements [3, data not shown] [3].

2.3. Thylakoid isolation and pH treatment

Isolated intact Arabidopsis WT and trol chloroplasts were subsequently lysed by incubating in 10 mM Hepes/KOH pH 7.6 and 5 mM MgCl₂, for 30 min on ice. Thylakoids were separated from stroma by centrifugation for 20 min at 50,000g, at 4°C, and treated by using different pHs. Thylakoids corresponding to 20 μg chlorophyll were re-buffered in 100 mM sodium phosphate buffer (pH 6.0, 7.0 or 8.0), 10 mM MgCl₂, 1 mM EDTA, in the absence of the detergents, or in the presence of 0.1% β-DM (v/v) or 0.1% β-DDM (v/v), and incubated for 10 min on ice, followed by additional 30 min at 25°C, as described [4]. Subsequently, samples were separated by ultracentrifugation for 20 min at 50,000g at 4°C into insoluble and soluble fractions, resolved by SDS-PAGE, followed by Western transfer and immunodecorated with the FNR antibody (as well as the α-AtpB and the α-Lhca2, as loading and extraction controls). Final detection was carried out by enhanced chemiluminescence (ECL) (Fig. 1). Negatives were scanned with the Hewlett-Packard flatbed scanner and the band intensities were quantified by using ImageJ program (NIH, USA).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2016.02.044.

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