Small chloroplast-targeted DnaJ proteins are involved in optimization of photosynthetic reactions

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Introduction

- DnaJ proteins, also called J-domain proteins, function as molecular co-chaperones of Hsp70 and play an important role in protein folding, unfolding, and assembly under both normal and stress conditions.
- Three DnaJ proteins, At1g80920, At4g36040 and At4g13830, or AtJ8, AtJ11 and AtJ20, are small chloroplast-targeted DnaJ proteins in *Arabidopsis* with predicted molecular masses of 18.3-, 17.8- and 23.4-kD, respectively. They belong to the simplest group of the DnaJ proteins, characterized by only one specific domain, the J-domain.
- To get more insights into the function of these small DnaJ proteins, the T-DNA insertion knockout mutants for AtJ8, AtJ11 and AtJ20 proteins, hereafter referred to as *j8*, *j11* and *j20*, respectively, were isolated and characterised.

Characterization of the *dnaj* mutants

Figure 1: Images of 4-week old wild-type (WT) plants and *j8*, *j11* and *j20* mutants.

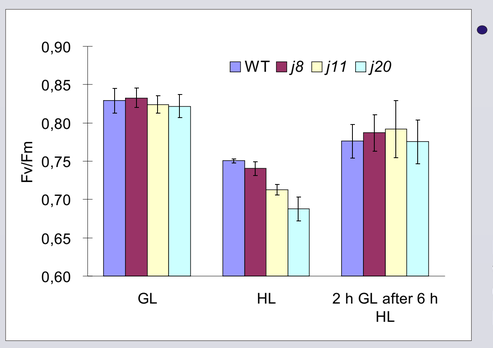
- Under standard growth conditions, the *dnaj* mutants did not exhibit significantly different phenotypes compared to wild-type (WT) except for slightly stunted growth of the *j11* and *j20* mutants (Figure 1).
- No difference was found in total chlorophyll amount per leaf area, in chlorophyll a or b contents nor in the Chl a/b ratio between the *dnaj* mutants and the WT (Table 1).

Table 1: Contents of leaf chlorophyll in WT and the *dnaJ* mutants under standard growth light condition (120 µmol photons m^{-2} s^{-1}), means ± SD (n = 10).

		Chl a+b (µg / cm^2)	Chl a/b	Chl a (µg / cm^2)	Chl a (µg / cm^2)
WT		18.80 ±0.91	3.57 ±0.05	14.69 ±0.73	4.11 ±0.18
j8	*salk_024617*	19.22 ±0.61	3.62 ±0.05	15.06 ±0.46	4.16 ±0.15
j11	*salk_015630*	18.55 ±0.49	3.57 ±0.05	14.49 ±0.40	4.06 ±0.11
j20	*salk_134365*	17.95 ±1.00	3.54 ±1.00	13.99 ±0.77	3.96 ±0.24

The *dnaj* mutants are suspectible to high light

- Photochemical efficiency of photosystem II (PSII) (F_v/F_m ratio) was not different between the WT and the *dnaj* mutants under growth light (GL) conditions, whereas it decreased somewhat more drastically in the mutants after exposure of 6 h to high light (HL), especially in *j11* and *j20* as compared to that in WT.



- When plants were returned to GL conditions, the PSII photochemical efficiency recovered quickly and no differences were found between the *dnaj* mutants and WT.

Figure 2: PSII photochemical efficiency of the *dnaJ* mutants. The values are means ± SD (n = 10) of ten independent experiments. GL, growth light (120 µmol photons m^{-2} s^{-1}); HL, high light (1000 µmol photons m^{-2} s^{-1}).

The *dnaj* mutants possess lower CO_2 assimilation capacity as compared to WT

- To analyse whether the DnaJ proteins are involved in acquiring the maximal CO2 fixation capacity, we measured both the light response and CO2 response curves of the *dnaJ* mutants and WT.

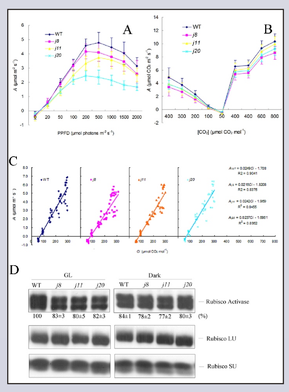
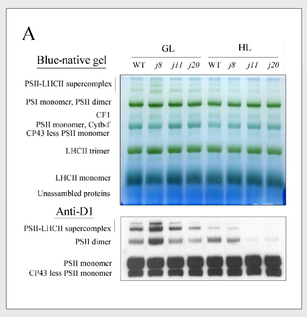


Figure 3: A, Light response curves; B, CO_2 response curves; C, A-Ci curves D, Immunoblot analysis of Rubisco Activase, Rubisco large (LU) and small subunit (SU) (mean ± SD, n=3).

- All three *dnaj* mutants suffered from limitation of the *in vivo* photosynthesis.
- The A-Ci curves confirmed that particularly the activity of Rubisco is compromised in the *dnaJ* mutants.
- Moreover, the amount of Rubisco Activase was lower in the mutants, suggesting that the three small chloroplast DnaJ proteins could be involved in the folding, unfolding, or assembly processes of this enzyme and thus participate in regulation of Rubisco activity.

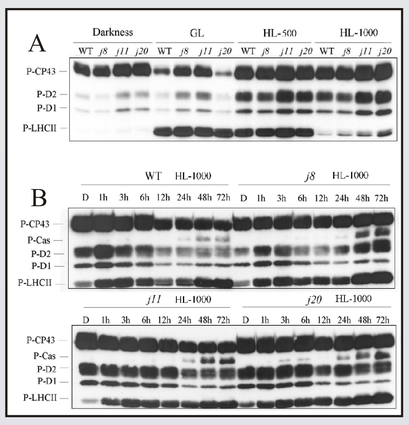
The DnaJ proteins provide stability for the PSII protein complexes



- The amount of PSII supercomplexes and dimers was lowered in the *dnaJ* mutants, especially in *j11* and *j20,* after 6h HL illumination (Figure 4).
- As the total amount of PSII was not lowered, it can be deduced that the three DnaJ proteins provide stability for the PSII protein complexes.

Figure 4: BN-PAGE analysis of thylakoid protein complexes from WT and the *dnaJ* mutants.

Knocking out one of the small chloroplast-targeted DnaJ proteins modifies the capacity for dynamic regulation of chloroplast redox reactions



- The *dnaJ* mutants, particularly *j11* and *j20*, accumulated more phosphorylated thylakoid proteins during different short-term illumination conditions (Figure 5A).
- During long-term HL illumination the *j11* and *j20* mutants kept the PSII core proteins strongly phosphorylated during the entire treatment, indicating lowered capacity for acclimation of the thylakoid redox reactions to prolonged exposure to HL (Figure 5B).

Figure 5: Thylakoid protein phosphorylation (A) after 6 h treatment under different light conditions and (B) during long-term high light treatment. 1.0 µg of Chl was loaded for immunoblotting with the P-Thr antibody. GL, 120 µmol photons m^{-2} s^{-1} ; HL-500, 500 µmol photonsm^{-2} s^{-1}, HL-1000, 1000 µmol photons m^{-2} s^{-1}

The lack of the small chloroplast-targeted DNAJ proteins triggers a global stress response

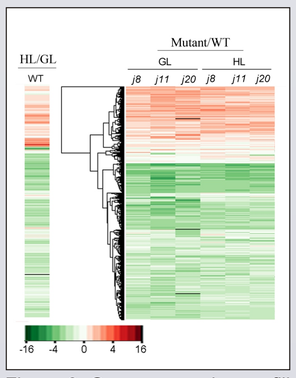


Figure 6: Gene expression-profilings of the DnaJ mutants with comparison to WT. Genes, whose expression showed more than a two-fold change, are shown (red: upregulated; green, down-regulated; black, missing value).

- All the *dnaj* mutants up-regulated the expression of several genes encoding ROS-detoxifying enzymes (e.g. *Apx6*, *Cat1*, *CSD3*, *Gpx5*) and stress-related transcription factors even under normal GL conditions to similar extent as the WT plants exposed to HL (Figure 6). Thus, the gene expression profiles of the *dnaJ* mutants under GL conditions resembled to those induced upon HL treatment in WT.

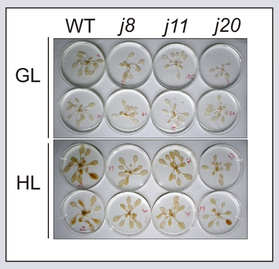


Figure 7: Histochemical detection of H_2O_2 with DAB staining after 6 h incubation of leaves under GL and HL.

- The upregulation of ROS scavenging and antioxidant genes coincided with lower H_2O_2 content in the *dnaJ* mutants than in WT, both at GL and HL conditions (Figure 7). In addition, the contents of several antioxidant proteins were higher in the *dnaj* mutants (data not shown).

- These results strongly indicate that a global stress response has been triggered in the *dnaJ* mutants even in the absence of external stress.

Conclusions and future plans

- It is conceivable that the tolerance of the dnaJ mutants to oxidative stress results from an unbalance of the redox reactions in chloroplasts, thereby modifying the chloroplast retrograde signaling mechanisms and inducing the up- or down-regulation of stress responsive genes in the nucleus.
- As a whole, it can be concluded the tolerance of the *dnaJ* mutant plants to oxidative stress occurs at the expense of the flexibility of photosynthetic reactions.
- Further studies with double and triple mutants are expected to provide stronger phenotypes and also to give deeper insights into the functions of the AtJ8, AtJ11 and AtJ20 proteins.
- I will also study the protein-protein interactions and search for the interaction partners for the DNAJ proteins. In addition, to achieve holistic information concerning the adaptive flexibility of the photosynthetic machinery, I will assess the importance of the DNAJ proteins - and indirectly, the importance of the photosynthetic processes in which they are functioning - by studying the fitness of the *dnaj* mutant plants in field conditions at the University of Umeå, Sweden.

Literature:

Chen, K., Holmström, M., Raksajit, W., Suorsa, M., Piippo, M., Aro, EM. 2010. Small chloroplast-targeted DnaJ poteins are involved in optimization of photosynthetic reaction in *Arabidopsis thaliana*. BMC Plant Biology, in press.