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## Disturbed $H^+/e^-$ ratio in *Arabidopsis* mutant *pgr6* defective in cytochrome *bf* complex activity

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### Introduction

Proper ATP/NADPH ratio required in chloroplasts is influenced by changes in metabolism, such as enhanced photorespiration. The ratio is optimized by the possible regulation of the transmembrane proton gradient formation per an electron passing through electron transport pathway ( $H^+/e^-$  ratio).

Q cycle, which was originally proposed in cytochrome (Cyt) *bc<sub>L</sub>* complex (Mitchell 1976), can function to increase efficiency of proton uptake to electron transfer in Cyt *bf* complex. In the theory of Q cycle, one electron from plastoquinol binding to Qo site reduces the Rieske Fe-S center and then Cyt *f*, which is oxidized by plastocyanin. Another electron from plastoquinol reduces Cyt *b* which is oxidized by plastoquinone at Qi site near stromal side. The cycle functions to incorporate additional protons from the stroma. By constitutive operation of Q cycle,  $H^+/e^-$  ratio increase 1.5 times. In addition to Q cycle, cyclic electron flow around PSI and water-water cycle also have been believed to contribute to attain theoretical NADPH/ATP ratio (Hope 1993). Molecular identity of the regulation and its physiological significance has been unclear.

### Materials and methods

#### *Plant materials and growth conditions*

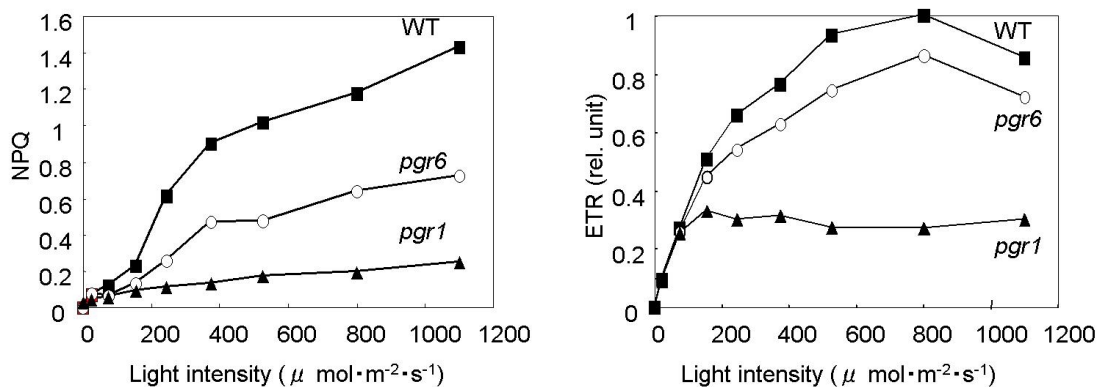
*Arabidopsis thaliana* (ecotype Columbia *gll*) seedlings were cultured in soil under culture room conditions ( $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ , 16 h-light / 8 h-dark cycles at 23°C). *pgr6* and *pgr1* were previously referenced as CE11-8-1 and CE10-10-1, respectively (Shikanai et al. 1999). Plants grown for 3 weeks after the germination were used for measurements.

#### *Chlorophyll fluorescence measurement*

*In vivo* Chlorophyll (Chl) *a* fluorescence was excited and detected using the PAM (pulse amplitude modulation) Chl fluorometer (PAM101, 103, Walz, Effeltrich, Germany). The minimum Chl fluorescence at the open PSII center ( $F_o$ ), was determined by measuring light (650nm) at a light intensity of  $0.05\text{--}0.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ . A saturating pulse of white light (800 ms,  $3,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) was applied to determine  $F_m$ . Actinic light ( $23\text{--}1,100 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) was applied to determine the NPQ  $\{(F_m - F_m')/F_m'\}$  and relative ETR  $\{(F_m' - F_s)/F_m' \times \text{actinic light intensity} (\mu\text{mol m}^{-2} \text{s}^{-1})\}$ , where  $F_m'$  is the maximum Chl fluorescence in the light (Genty et al. 1989, Schreiber 1986).

## Results and Discussion

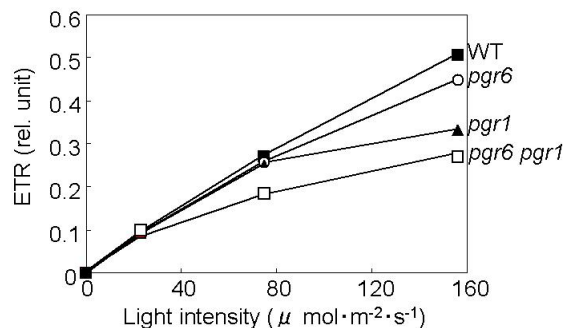
An *Arabidopsis* mutant, *pgr6* was identified by the reduced NPQ using Chl fluorescence imaging system (Shikanai et al. 1999). Although the Chl content was unaffected, the growth rate was slightly reduced under photoautotrophic culture conditions in *pgr6*. Fig. 1a shows the light-intensity dependence of NPQ in *pgr6*, *pgr1* and the wild type. Due to the specific mutation in the Rieske Fe-S subunit of Cyt *bf* complex, NPQ induction was severely affected in *pgr1* (Munekage et al. 2001). In the wild type, NPQ increased in the course of increase in light intensity. In contrast to *pgr1*, *pgr6* exhibited induction of NPQ, which was depending on light intensity. However, the extent of NPQ was inferior to that in the wild type. Fig. 1b shows the light-intensity dependence of ETR. In the wild type ETR was saturated at light



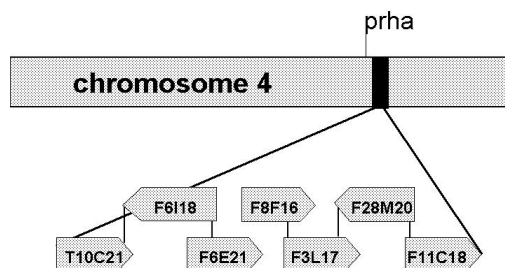
**Figure 1.** Light-intensity dependence of NPQ (a) and ETR (b). WT and *pgr6* was irradiated with excitation light for 4 minutes, respectively. ETR is represented as relative values.

intensity of  $800\ \mu\text{mol}\ \text{m}^{-2}\ \text{s}^{-1}$ . On the other hand, ETR was saturated at much lower light intensity ( $150\ \mu\text{mol}\ \text{m}^{-2}\ \text{s}^{-1}$ ) with the lower level (33%) in *pgr1*. The *pgr1* phenotype is explained by the defect in Rieske subunit, which restricts electron transport only at high light intensity (Munekage et al. 2001). In *pgr6*, however, ETR was only mildly affected comparing with significant reduction in NPQ.

To assess the possibility that *pgr6* has a defect in Cyt *bf* complex that is a major site of pH generation, ETR was analyzed in a double mutant *pgr6pgr1*. Although double mutant exhibited the same NPQ induction pattern with *pgr1* (data not shown), ETR was severely affected even at lower light intensity (Fig. 2).



**Figure 2.** Double mutant exhibited the synergistic phenotype in ETR. Light intensity-dependence of ETR in *pgr6*, *pgr1*, *pgr6 pgr1*.



**Figure 3.** Map position of *pgr6*

The results suggest that *pgr6* and *pgr1* mutations influenced the phenotype synergistically, indicating that the *pgr6* defect is also related with Cyt *bf* complex. Western analysis of Cyt *f* did not show any difference of the protein level between *pgr6* and the wild type, indicating that the *pgr6* defect is qualitative as is in *pgr1*. By chromosome mapping, the *pgr6* locus was specified in the region about 800kb near the molecular marker *prha* on the 4th chromosome (Fig. 3). In this region, no gene was found to encode any Cyt *bf* complex subunit respected. *PGR6* may encode a novel Cyt *bf* complex subunit, or the protein functioning in regulation of Cyt *bf* complex activity.

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