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An *Arabidopsis thaliana* high Fo mutant had an impaired 33 kDa protein in oxygen evolving complex (OEC)

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Introduction

High chlorophyll fluorescence has been used to monitor the impairment in photosynthetic activity. (Shikanai et al. 1998). These mutants could be classified into three classes, photochemical quenching mutants, non-photochemical quenching mutants and high Fo mutants (Shikanai et al. 1999). The photochemical quenching mutants have a defect in one of various proteins which consist of or assist photosynthetic electron transport. The non-photochemical quenching mutants are defective in wasting excessive energy as heat. The third group, high Fo mutants have a defect in oxidizing side of P680 (Shreiber and Neubauer 1987) or in general gene expression in plastid (Balkan and Goldschmidt 2000), resulting in photoinhibition of PS II.

Thus, we screened high Fo mutants to isolate the mutants with defects in oxygen evolving complex (OEC) in PSII. Among several high Fo mutants one showed low accumulation of OEC 33kDa protein (OEC33). We report some characterization of this high Fo mutant, *hFo1*.

Materials and methods

Plant materials

Seeds of wild type and high Fo mutants (Shikanai et al. 1999) were sown in soil after cold treatment for 24 hour at 4˚C. They were grown at 23˚C under white light (50~100 μmol /m^2s with the light/dark cycle of 9/15 h.

Protein analysis

Arabidopsis thaliana leaves were frozen in liquid nitrogen, homogenized for SDS-PAGE analysis. Proteins were made soluble in the sample buffer (125mM Tris-HCl pH6.8, 20% glycerol, 5% SDS and bromophenolblue), and analyzed on a 12.5% polyacrylamide gel containing SDS and no urea. Proteins from homogenized leaves were made soluble in 0.8 M Tris-HCl (pH 8.3) in dark at 4˚C, and equal volume of sample buffer were added for native-PAGE (125 mM Tris-HCl pH 6.8, 20% glycerol and bromophenolblue) for immuno-blot analysis. In both analysis proteins were extracted and analyzed on the basis of the same fresh weight.

Measurement of chlorophyll fluorescence patterns

Induction patterns of chlorophyll fluorescence were measured under low light (30 μmol / m^2 s) and high light (1000 μmol / m^2 s) with a PAM2000 on intact leaves after 30 min dark

adaptation (Waltz). Fm and Fm' were measured by application of a 1-s pulse of saturating white light.

Results

Protein analysis

High Fo mutants have either mutation in the oxidizing side of P680 or mutation in plastid gene expression. To isolate OEC mutants, we measured the amounts of OEC23 or OEC33 in high Fo mutants by immuno-blot analysis. Among 20 high Fo mutants analyzed, a mutant (*hFo1*) showed considerably low amount of OEC33. More careful immuno-blotting analysis showed that wild type had two OEC33 bands, whereas *hFo1* lacked a band with fast mobility. CBB staining also showed the difference of the protein quantity near the 33kDa between the wild type and *hFo1* (Fig.1).

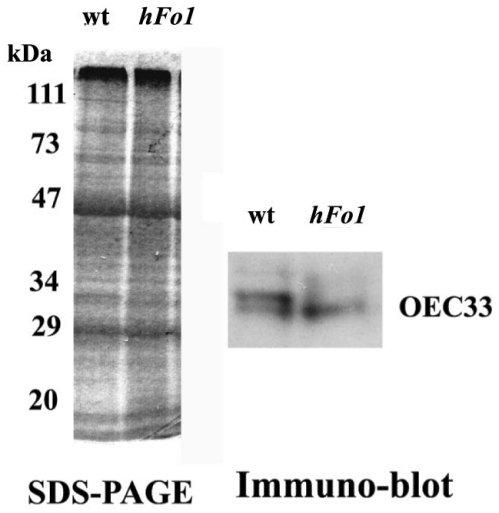

Figure 1 CBB-staining and immuno-blot of wild type(wt) and a high Fo mutant (*hFo1*).

Sequencing analysis of psbO *and* psbO2

Two genes, *psbO* and *psbO2*, have been reported to encode OEC33 in *Arabidopsis thaliana* (Kieselbach et al. 2000). Immuno-blot analysis suggested the possibility that one of OEC33 genes was missing in *hFo1*. To examine this possibility, *psbO* and *psbO2* genes were amplified by PCR and subcloned. Sequence analysis showed that *psbO* gene of the mutant had a substitution of C for T at the 159th amino acid Gln. The substitution changed 159th Gln codon to stop codon. On the other hand no change in *psbO2* was found in wild type and *hFo1*.

Induction patterns of chlorophyll fluorescence

Since a mutation in *psbO* was confirmed, we re-examined the induction patterns of *hFo1*. The potential quantum yield of PSII, expressed as (Fm-Fo)/Fm, was much lower in the mutant (0.5~0.6) than that in wild type (about 0.8). This lower (Fm-Fo)/Fm in the mutant was apparently due to high Fo (Fig. 2). Actual quantum yield of PSII (.F/Fm') in either low or high light was also lower in the mutant than that in wild type. These results suggested that the mutation in *psbO* resulted in low photosynthetic ability and limited the growth; the leaf size of the mutant was smaller than wild type in their all growth stage.

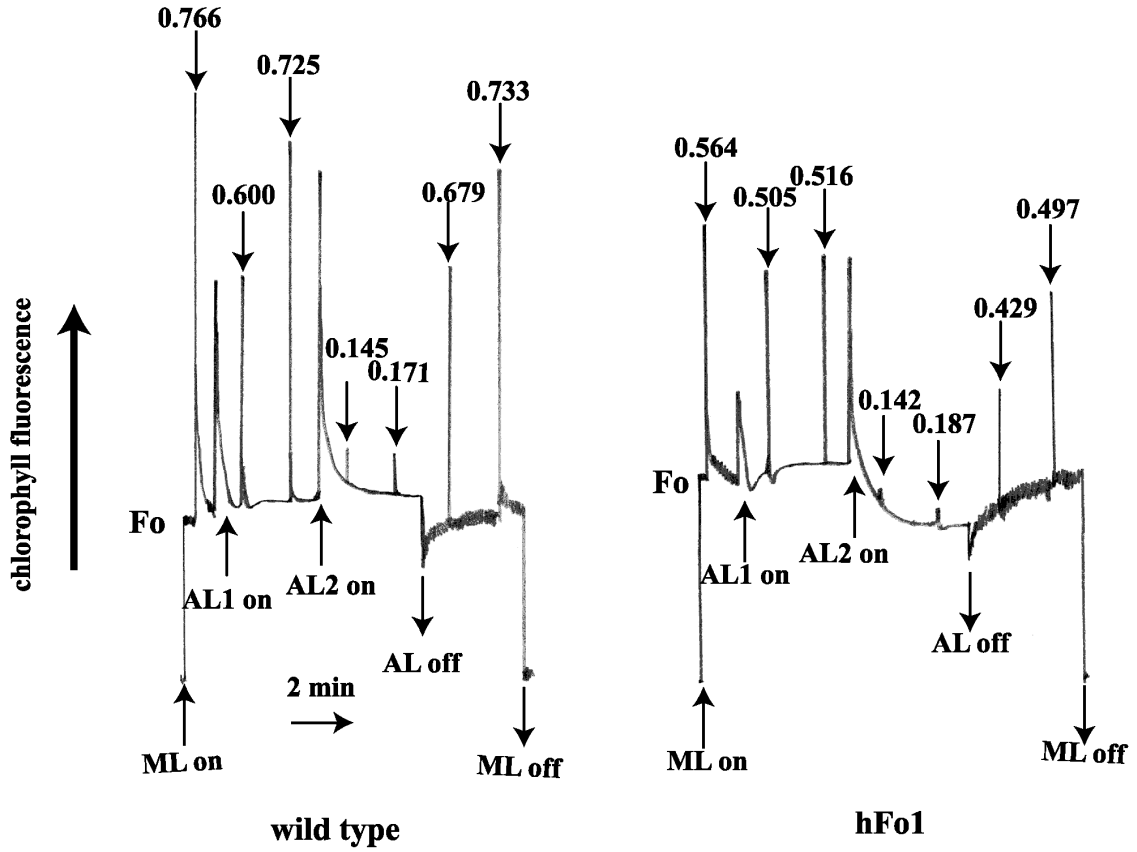

Figure 2 The fluorescence patterns measured with a PAM2000 (Waltz) after 30 min dark adaptation. AL(actinic light)1 is the start of the low light irradiation (30 μmol / m^2 s) and AL2 is the start of high light irradiation (1000 μmol / m^2 s). Downward arrows show the 1-s flash of the saturation pulse. Values above the arrows are quantum yield of PSII (ΔF/Fm'). ML is the measuring light.

The separation of phenotype

To confirm that the high Fo phenotype was derived from the single mutation in *psbO*, we carried out the genetic analysis of mutant. The first generation (F1) of hybrid of wild type and the mutant showed all wild type phenotype. And F2 showed 16 wild type phenotype plants and 5 mutant phenotype plants. The about 3:1 ratio of wild type and mutant phenotype in F2 generation suggested that the mutation was single and recessive.

Discussion

Sequencing analysis suggested that an introduction of stop codon resulted in the decrease in OEC33 proteins as shown by SDS-PAGE and immuno-blot analysis. However the exact molecular mechanism of low quantum yield of PSII in *hFo1* is not clear because the mutant still has complete *psbO2* gene and gene product. Wild type phenotype found in the hybrid of wild type and *hFo1* suggested that the total amount of OEC33 protein is important or OEC33 translated from a single *psbO* gene might be more efficient for the PSII activity. More detailed analysis is needed to determinate the functional differences between *psbO* and *psbO2* genes.

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