Analysis of PSII antenna size heterogeneity of *Chlamydomonas reinhardtii* during state transitions

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State transition:
- migration of LHCII pigment-proteins between PSII and PSI
- up to 80% in *Chlamydomonas reinhardtii* (Delosme et al. 1996)

Different types of PSII with different antenna sizes: PSII antenna size heterogeneity (Melis and Homann (1975))
Fluorescence rise from $F_O$ to $F_M$ corresponding to the reduction of $Q_A$ in the reaction center of PSII.

- DCMU addition $\rightarrow$ the photochemical phase
- DCMU fluorescence rise induction kinetic is not a first order kinetic $\rightarrow$ PSII$\alpha$ and PSII$\beta$
Lavergne et al. (2004):

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<th>PSIIα</th>
<th>PSIIβ</th>
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<tr>
<td>Proportion</td>
<td>+</td>
<td>-</td>
</tr>
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<td>Antenna</td>
<td>210-250 Chl</td>
<td>≈ 100 Chl</td>
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<td>Region of the thylakoïd membrane</td>
<td>appressed</td>
<td>non appressed</td>
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Connectivity (p) : quantifies the probability of energy transfer between closed PSII to an open PSII (Joliot and Joliot, 1964)
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Purpose of this work : determination of PSII antenna size heterogeneity in state I and in state II

Method of Melis and Homann (complementary area over the DCMU-FR) is very approximative due to approximations in the $F_M$ level

Non linear regression algorithm with equations derived from Lazár et al. (2001):
- better $F_M$ determination
- $p$ determination
- simultaneous fitting of several curves

$$rF_V(t) = \sum_{i=1}^{3} \frac{(1-p_i)\text{PSII}_{i}^{closed}(t)}{1-p_i\text{PSII}_{i}^{closed}(t)}$$

$$\text{PSII}_{i}^{closed}(t) = \text{PSII}_{i,0}^{open}(1 - e^{-k_i t})$$
Experiments

Choice of the model

1. \[ rF_V(t) = \%\text{PSII}_\alpha \frac{(1-p\alpha)(1-e^{-k\alpha t})}{1-p\alpha(1-e^{-k\alpha t})} + \%\text{PSII}_\beta (1-e^{-k\beta t}) + \%\text{PSII}_\gamma (1-e^{-k\gamma t}) \]

2. \[ rF_V(t) = \%\text{PSII}_\alpha \frac{(1-p\alpha)(1-e^{-k\alpha t})}{1-p\alpha(1-e^{-k\alpha t})} + \%\text{PSII}_\beta \frac{(1-p\beta)(1-e^{-k\beta t})}{1-p\beta(1-e^{-k\beta t})} + \%\text{PSII}_\gamma (1-e^{-k\gamma t}) \]

3. \[ rF_V(t) = \%\text{PSII}_\alpha \frac{(1-p\alpha)(1-e^{-k\alpha t})}{1-p\alpha(1-e^{-k\alpha t})} + \%\text{PSII}_\beta \frac{(1-p\beta)(1-e^{-k\beta t})}{1-p\beta(1-e^{-k\beta t})} + \%\text{PSII}_\gamma \frac{(1-p\gamma)(1-e^{-k\gamma t})}{1-p\gamma(1-e^{-k\gamma t})} \]

The Akaike’s information criterion (AIC) : comparison of different models by introducing a penalty for the number of parameters used.

\[ AIC = 2k - 2\log(L) \]

<table>
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<th>model</th>
<th>AIC</th>
</tr>
</thead>
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<tr>
<td>1: connectivity allowed only for PSII\alpha</td>
<td>-14599</td>
</tr>
<tr>
<td>2: connectivity allowed for PSII\alpha and PSII\beta</td>
<td>-17025</td>
</tr>
<tr>
<td>3: connectivity allowed for PSII\alpha, PSII\beta and PSII\gamma</td>
<td>-17007</td>
</tr>
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</table>

- \( \text{AIC}_{\text{model2}} < \text{AIC}_{\text{model3}} < \text{AIC}_{\text{model1}} \) → model 2 describes the experimental data better than models 1 and 3
- connectivity for PSII\beta \neq 0, in contrast with a majority of studies (except the work of Lavergne and Trissl (1995) and Lazár et al.(2001))
Experiment:

- Darkness 1 hour $\rightarrow$ Oxidation of plastoquinones $\rightarrow$ Algae close to state I
- Arrest of mitochondrial respiration $\rightarrow$ Reduction of plastoquinones $\rightarrow$ Transition to state II

When PQ pool is highly reduced (state 2) $\rightarrow$ many PSII centers have a $Q_B^-$ bound and the addition of DCMU leads to $Q_A$ reduction before the illumination.
PQ pool had to be rapidly oxidized before the addition of DCMU $\rightarrow$ development of a method with N$_2$ bubbling

- to monitor the redox state of Q$_A$ without the influence of state transitions $\rightarrow$ mutant stt7
- Complete reoxydation of PQH$_2$ by O$_2$ in 2 minutes
- In 2 minutes, back transition to state 1 is not significant in the wt $\rightarrow$ ideal delay
Experiments

PSII heterogeneity during state transition

The conversion of PSII\(_\alpha\) to PSII\(_\beta\) during transition from state 2 to state 1 parallels the decrease of the low T fluorescence ratio.

State transitions can be described as changes in the proportions of two PSII populations with constant properties.

<table>
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<tr>
<th></th>
<th>%PSII(_\alpha) S1</th>
<th>%PSII(_\alpha) S2</th>
<th>(p_\alpha)</th>
<th>(p_\beta)</th>
<th>(k_\alpha) (s(^{-1}))</th>
<th>(k_\beta) (s(^{-1}))</th>
<th>(\frac{k_\alpha}{k_\beta})</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>0.48</td>
<td>0.16</td>
<td>0.62</td>
<td>0.15</td>
<td>48.6</td>
<td>15.4</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Thomas de Marchin (lab of bioenergetics, ULg)
In addition to this description of heterogeneity by functional analysis of the fluorescence rise of PSII *in vivo*: biochemical studies (mainly based on isolation of PSII complexes and subsequent analysis).

Iwai et al., 2008

We suggest that PSII*α* phase refers to PSII mega- and super- complexes and that PSII*β* phase refers to PSII core complexes.
Summary:

- development of a protocol for PSII heterogeneity analysis during state transition and improvement of mathematical analysis
- connectivity for PSII$_\beta$
- demonstration of an interconversion of PSII$_\alpha$ to PSII$_\beta$ during state transitions for the first time \textit{in vivo}
- link between functional approach and biochemical and structural studies
Summary:

- Development of a protocol for PSII heterogeneity analysis during state transition and improvement of mathematical analysis
- Connectivity for PSIIβ
- Demonstration of an interconversion of PSIIα to PSIIβ during state transitions for the first time *in vivo*
- Link between functional approach and biochemical and structural studies

Thank you for your attention
\[
\begin{align*}
 rF_V(t) & = \sum_{i=1}^{3} \frac{(1-p_i)\text{PSII}_{i}^{\text{closed}}(t)}{1-p_i \text{PSII}_{i}^{\text{closed}}(t)} \\
 \text{PSII}_{i}^{\text{closed}}(t) & = \text{PSII}_{i,0}^{\text{open}} \left(1 - e^{-k_i t}\right) \\
 k_i(t) & = \frac{k_i^0}{1-p_i \text{PSII}_{i}^{\text{closed}}(t)} \\
 k_i^0 & = \frac{1-p_i}{t(p_i rF_V,i(t) + 1 - p_i)} \left( \ln \left(1 - p_i \left(1 - rF_V,i(t) \right) \right) - \ln \left(1 - rF_V,i(t) \right) - \ln \left(1 - p_i \right) \right)
\end{align*}
\]