Biochimica et Biophysica Acta 1837 (2014) 1548–1552

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Repressible chloroplast gene expression in *Chlamydomonas*: A new tool for the study of the photosynthetic apparatus ☆

Emine Dinc [a], Silvia Ramundo [b], Roberta Croce [a], Jean-David Rochaix [b,*]

[a] Department of Physics and Astronomy, Faculty of Sciences, VU University Amsterdam, 1081 HV Amsterdam, The Netherlands
[b] Departments of Molecular Biology and Plant Biology, University of Geneva, 30, Quai Ernest Ansermet, 1211 Geneva, Switzerland

ARTICLE INFO

Article history:
Received 7 October 2013
Received in revised form 26 November 2013
Accepted 29 November 2013
Available online 12 December 2013

Keywords:
Chloroplast
Gene expression
Photosystem degradation
Chlamydomonas

ABSTRACT

A repressible/inducible chloroplast gene expression system has been used to conditionally inhibit chloroplast protein synthesis in the unicellular alga *Chlamydomonas reinhardtii*. This system allows one to follow the fate of photosystem II and photosystem I and their antennae upon cessation of chloroplast translation. The main results are that the levels of the PSI core proteins decrease at a slower rate than those of PSII. Amongst the light-harvesting complexes, the decrease of CP26 proceeds at the same rate as for the PSII core proteins whereas it is significantly slower for CP29, and for the antenna complexes of PSI this rate is comprised between that of CP26 and CP29. In marked contrast, the components of trimeric LHCII, the major PSII antenna, persist for several days upon inhibition of chloroplast translation. This system offers new possibilities for investigating the biosynthesis and turnover of individual photosynthetic complexes in the thylakoid membranes. This article is part of a Special Issue entitled: Photosynthesis Research for Sustainability: Keys to Produce Clean Energy.

1. Introduction

The primary reactions of photosynthesis are catalyzed by three major protein–pigment complexes which are serially connected by the photosynthetic electron transport chain. They include photosystem II (PSII), the cytochrome b_6f complex (Cytb_6f) and photosystem I (PSI). Both PSII and PSI are associated with their own light-harvesting complexes LHCII and LHCI, respectively, and catalyze the primary photochemical reactions in which the absorbed light energy is used to induce a stable charge separation across the thylakoid membrane. The donor side of PSII creates a strong oxidant capable of extracting electrons from water with their subsequent transfer along the electron transport chain to plastoquinone, Cytb_6f and PSI. The acceptor side of PSI creates in turn a strong reductant capable of reducing ferredoxin and finally, through ferredoxin-NADP reductase (FNR), $NADP^+$ to NADPH. This electron flow is coupled to proton transfer from the stroma to the lumen side of the thylakoid membrane and the resulting proton motive force is used by the ATP synthase to generate ATP. Finally, both ATP and NADPH act as energy source and reducing power, respectively, for driving CO_2 assimilation by the Calvin–Benson cycle.

The unicellular alga *Chlamydomonas reinhardtii* has emerged as a powerful model system for studying chloroplast function, biogenesis and regulation for several reasons [1]. First, its photosynthetic function is dispensable, provided a source of reduced carbon such as acetate is present in the growth medium. It is thus easy to isolate, maintain and study mutants deficient in photosynthetic activity either in the dark (heterotrophic conditions) or in the light (mixotrophic conditions). Second, this alga is able to synthesize chlorophyll in a light-independent manner and can thus accumulate a fully functional photosynthetic apparatus when grown in the dark. This feature is particularly important as mutants deficient in photosynthesis are often light-sensitive and difficult to study in land plants. Third, transformation of this alga is possible for the nuclear, chloroplast and mitochondrial compartments. Finally, the nuclear, chloroplast and mitochondrial genomes have been sequenced and can easily be screened for the presence of specific genes [2–4].

Chloroplast genomes encode between 100 and 120 genes in green algae and land plants. These genes can be grouped in three major classes. The first includes genes coding for components of the photosynthetic machinery, the second comprises genes involved in chloroplast gene expression such as the subunits of the plastid RNA polymerase and ribosomal proteins, and the third includes genes involved in various chloroplast functions as well as some genes of unknown function. Because homologous recombination occurs in the chloroplast, it is possible to perform site-specific changes of plastid genes of *C. reinhardtii* through chloroplast transformation [5]. Usually the bacterial *aadA* gene conferring resistance to the antibiotics spectinomycin and streptomycin is used for selection of the transformants [6]. In this way either chloroplast

☆ This article is part of a Special Issue entitled: Photosynthesis Research for Sustainability: Keys to Produce Clean Energy.

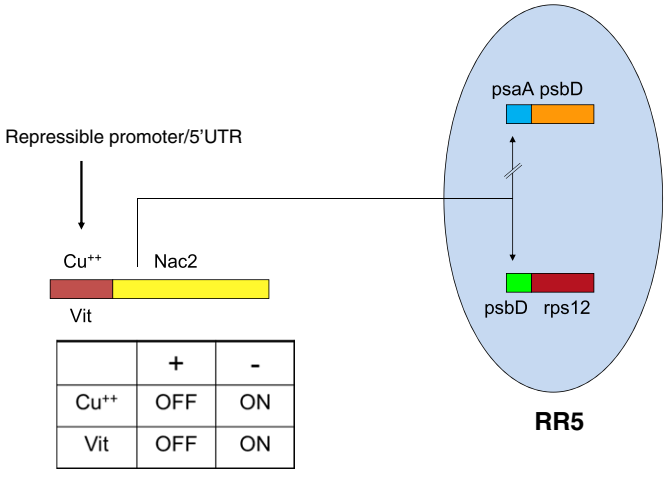
* Corresponding author. Tel.: +41 22 379 6187.
E-mail address: Jean-David.Rochaix@unige.ch (J.-D. Rochaix).

0005-2728/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbabio.2013.11.020

gene disruptions or site-specific mutagenesis can be achieved. However this is only possible if the genetic changes do not compromise cell viability. Chloroplast genomes are polyploid and in the particular case of *C. reinhardtii* there are 80 copies of the chloroplast genome per chloroplast or cell as this alga contains a single chloroplast. If all copies of a particular chloroplast gene can be disrupted, i.e. if a homoplasmic state is achieved, under hetero- or mixo-trophic growth conditions, this indicates that this gene does not have an essential function. This applies in particular for genes involved in photosynthesis. If however the gene one attempts to inactivate has an essential function it is not possible to achieve homoplasmicity and, as long as the selection for antibiotic resistance is maintained, a heteroplasmic state persists with a mixed population of intact and disrupted genes.

The photosynthetic complexes have a dual genetic origin as their subunits are encoded both by the chloroplast and nuclear genomes. Their assembly thus depends on a concerted interplay between these two genetic systems. Genetic analysis of the biogenesis of the photosynthetic apparatus in the green alga *C. reinhardtii* and the land plants Arabidopsis and maize revealed a large number of nucleus-encoded factors which act as chloroplast trans-acting activators of chloroplast gene expression [7]. They are mostly involved in different post-transcriptional steps of chloroplast gene expression including RNA processing, RNA stability, splicing and translation. A remarkable feature is that several of these factors act in a gene-specific manner indicating that altogether hundreds of factors of this sort must be acting in the plastids as activators of gene expression. Another important point is that many of these factors interact specifically with the 5′untranslated region (5′UTR) of their plastid target gene. It is therefore possible to make the expression of a reporter gene dependent on one of these factors by fusing the reporter gene to the 5′UTR of the corresponding target gene (Fig. 1). We have exploited these unique features of plastid gene expression for developing a robust inducible and repressible chloroplast gene expression system in *C. reinhardtii* [8,9]. This system opens new interesting possibilities for the study of both basic and applied aspects of chloroplast biology and photosynthesis.

Because no tight repressible chloroplast promoter is available in *C reinhardtii*, the following strategy was used. We took advantage of our earlier studies on the nuclear gene Nac2 which is specifically required for the accumulation of the chloroplast *psbD* mRNA [10]. The Nac2 protein interacts with the *psbD* 5′UTR and thereby stabilizes the *psbD* mRNA. In the absence of Nac2 this mRNA is specifically degraded but all other plastid RNAs accumulate normally. To obtain repressible chloroplast gene expression, the Nac2 gene was fused to a repressible/inducible promoter, either the Cyc6 promoter of cytochrome *c6*, which is repressed by copper and expressed in its absence [9] or the MetE promoter and the Thi4 riboswitch, which are repressed in the presence of vitamins B12 and thiamine, respectively [8,11,12] (Fig. 1). Any chloroplast gene can be used as target gene, provided it is fused to the *psbD* 5′UTR, the target of Nac2. This system offers many possibilities for investigating problems related to the function and regulation of chloroplasts. First it allows one to progressively deplete essential proteins from the chloroplast and to study the effect of this depletion. This approach is particularly powerful for essential plastid genes of unknown function. Second, it allows one to remove specifically and in a reversible way any of the major photosynthetic complexes and to examine its biosynthesis in a preformed thylakoid membrane as well as the effect caused by its depletion on cellular metabolism. As an example, by depleting PSII, cells enter into an anaerobic state because of consumption of oxygen by respiration, a state which induces the production of hydrogen, at least transiently [9]. Third, this system allows one to transiently express foreign proteins of biotechnological interest which are toxic to the cells.



	+	-
Cu++	OFF	ON
Vit	OFF	ON

Fig. 1. Repressible chloroplast gene expression system. Scheme of the repressible chloroplast gene expression system. The nuclear Nac2 gene is fused either to the Cyc6 promoter which is repressed by copper or the MetE promoter and Thi4 riboswitch which are repressed by vitamin B12 and thiamine, respectively. The target of Nac2 is the *psbD* 5′UTR. In this particular case it was fused to the coding sequence of *rps12* making the expression of this gene dependent on Nac2 (strain RR5). The coding sequence of *psbD* was fused to the *psaA* 5′UTR to render *psbD* expression independent of Nac2 [8].

Here we describe and discuss this new approach and present one example in which the system can be used to follow the decline of the proteins of PSII and PSI and their associated light-harvesting complexes when chloroplast protein synthesis ceases. Although, protein turnover is normally studied through pulse and pulse-chase experiments with labeled methionine [13] or by using antibiotics which specifically inhibit translation [14], these methods are not suitable for the study of the light-harvesting complexes in *Chlamydomonas* for several reasons. First, protein labeling of *Chlamydomonas* with amino acids is only possible with labeled arginine but not with methionine although cells can be labeled with S35-sulfate or C14-acetate [1]. Second the very low turnover of the light harvesting systems makes the interpretation of pulse labeling and pulse-chase experiments difficult. Third, secondary effects of antibiotics cannot be excluded especially if they are used for long periods. Because repression of chloroplast gene expression occurs gradually in this system, it allows one to extend the time scale which is particularly useful when processes that occur sequentially in time are studied. We have taken advantage of our newly developed repressible chloroplast gene expression system for monitoring the fate of the core PSII and PSI subunits and of individual components of their light-harvesting complexes upon inhibition of chloroplast protein synthesis.

2. Material and methods

2.1. Strains and cell growth

The RR5 strain containing the Nac2 gene fused to the MetE promoter and Thi4A riboswitch [8] was grown in TAP medium [15] under continuous light (60 μmol photons m^{-2} s^{-1}). Addition of vitamins B12 and thiamine-HCl for Nac2 repression was done as described [8].

2.2. Determination of Chl content

The chlorophyll concentration was determined as described previously [16]. The values are averages ± SD of four measurements.

2.3. Fluorescence measurements

Maximum quantum efficiency of PSII (Fv/Fm) was measured with a chlorophyll fluorescence & P700 photosynthesis analyzer (Dual-PAM 100; Walz Instruments). Prior to each measurement, cells were dark-adapted for ~1 to 5 min.