Global spectroscopic analysis to study the regulation of the photosynthetic proton motive force: A critical reappraisal

Guillaume Allorent, Martin Byrdin, Luca Carraretto, Tomas Morosinotto, Ildiko Szabo, Giovanni Finazzi

ABSTRACT

Keywords:
- Electrochromic shift
- Proton motive force
- Ion channels
- Photosynthesis

In natural variable environments, plants rapidly adjust photosynthesis for optimum balance between photochemistry and photoprotection. These adjustments mainly occur via changes in their proton motive force (pmf). Recent studies based on time resolved analysis of the Electro Chromic Signal (ECS) bandshift of photosynthetic pigments in the model plant Arabidopsis thaliana have suggested an active role of ion fluxes across the thylakoid membranes in the regulation of the pmf. Among the different channels and transporters possibly involved in this phenomenon, we previously identified the TPK3 potassium channel. Plants silenced for TPK3 expression displayed light stress signatures, with reduced Non Photochemical Quenching (NPQ) capacity and sustained anthocyanin accumulation, even at moderate intensities. In this work we re-examined the role of this protein in pmf regulation, starting from the observation that both TPK3 knock-down (TPK3 KD) and WT plants display enhanced anthocyanin accumulation in the light under certain growth conditions, especially in old leaves. We thus compared the pmf features of young “green” (without anthocyanins) and old “red” (with anthocyanins) leaves in both genotypes using a global fit analysis of the ECS. We found that the differences in the ECS profile measured between the two genotypes reflect not only differences in TPK3 expression level, but also a modified photosynthetic activity of stressed red leaves, which are present in a larger amounts in the TPK3 KD plants.

1. Introduction

In chloroplasts, photosynthesis generates a proton motive force (pmf) thanks to the coupling between electron transfer in membranes (thylakoids) embedded complexes and proton release in the inner (lumen) compartment. The pmf not only comprises a $H^+$ gradient ($\Delta pH$), but also an electric field ($\Delta \Psi$), negative on the stromal side of the thylakoids. The $\Delta \Psi$ is generated by electron flow, due to the asymmetric location of the electron donors and acceptors of the different photosynthetic complexes. All the electron donors ($P_{680}$ and $P_{700}$, i.e. the primary donors to Photosystem II and I, respectively, plus the $Q_0/\text{Q}_b$ site of plastocyanin oxidation in cytochrome $b_{6}f$) are facing the luminal side of the thylakoids. All the electron acceptors (the $Q_a/\text{Q}_b$ plastoquinones in PSII, the phylloquinones/iron-sulphur clusters of PSI and the $Q_a/\text{Q}_b$ site of the cyt $b_{6}f$ complex) are facing the stromal side of the membranes [1]. Thus, light driven photosynthesis results in the accumulation of negative changes on the stromal side of the thylakoids, and of positive charges on the luminal side. Both components of the pmf equally contribute to ATP synthesis [2] for CO$_2$ assimilation and other metabolic activities, although the presence of a minimum $\Delta \Psi$ is required for ATP generation [3]. The $\Delta pH$ and $\Delta \Psi$ also play more specific roles. The proton gradient slows down the rate of electron transfer at the level of the cytochrome $b_{6}f$ complex. This phenomenon, known as the “photosynthetic control” [4], allows keeping the primary electron donor to $P_{680}$ in the oxidized state in the light [5], possibly to protect PSI against photoinhibition [6]. The $\Delta pH$ also modulates photoprotection of PSII, by enhancing thermal dissipation of light absorbed in excess with respect to the CO$_2$ assimilation capacity, in a process known...
as Non Photochemical Quenching (NPQ, reviewed in [7,8]). The ΔΨ has been recently suggested to modulate PSII photodamage, by controlling the rate of charge recombination within the complex and therefore singlet oxygen production by PSII [9]. The presence of a ΔΨ also affects the absorption characteristics of some photosynthetic pigments, leading to a shift of their absorption spectrum. This phenomenon, known as the electrochromic shift (ECS, [2]), can be used to monitor the pmf in vivo [10].

Because of the different roles of the two pmf components, their relative contribution to the pmf must be actively controlled. In mitochondria, a consensus exists on the notion that the ΔΨ is the predominant pmf component. Conversely, both the ΔΨ and the ΔpH accumulate in chloroplasts in the light, and the real partitioning of the pmf between its two components is still debated [11,12]. Experimental studies [13] and modelling [12] has led to the notion that it is possible to monitor the ΔpH/ΔΨ partitioning by following the time course of ECS changes during a light to dark transition. Typical kinetic of the ECS during the onset of illumination and in steady state conditions are presented in Fig. 1. During the dark to light transition, the ECS displays a complex kinetic behavior, which likely reflects changes in the turn-over rate of the photosynthetic complex (i.e. in the generation of the pmf by photosynthetic electron flow) and in ion flux (i.e. its dissipation via either H⁺ flux in the ATP synthase or counter ions movement [12]). After attainment of steady state, the ECS relaxes in the dark with a multiphasic kinetic. After the first relaxation phase (1), a slower ECS increase is seen (2), which has been previously interpreted as the signature of charge redistribution across membranes [13]. Finally, a pseudo steady state point is attained (3). There, the reversible part of the ECS signal (4) would correspond to the pmf stored as a ΔpH, while the irreversible one would correspond to the ΔΨ [13].

Recent studies have suggested that the presence of ion channels/transporters in the thylakoid membranes modulates the partitioning of ΔpH and ΔΨ under different conditions, thereby affecting the photosynthetic efficiency (reviewed in [14,15]). They include the potassium channel TPK3 [16], the K⁺/H⁺ antiporter KEA3 [17–19] and transporters of other charged species, including Cl⁻ [20–22], Mn²⁺ and Ca²⁺ [23,24]. Concerning TPK3, its ion specificity and function was assessed by electrophysiological experiments in planar lipid bilayers, revealing that it is a K⁺ selective channel belonging to the two-pore potassium channel family, regulated by Ca²⁺ and pH. Arabidopsis thaliana mutants fully silenced in the TPK3 gene displayed several phenotypes including altered thylakoid membrane organisation, lower efficiency of photosynthesis, higher susceptibility to photooxidation than WT plants and enhanced accumulation of anthocyanins already at 100 μmol photons m⁻² s⁻¹ light intensity [16]. Previous analysis of the kinetics of the ECS relaxation during a light-dark transition suggested that the pmf was differently partitioned in this mutant, with an increase in the ΔΨ at the expense of ΔpH [16]. TPK3 was thus proposed to play a role in counterbalancing proton influx into the lumen upon illumination. The change in pmf partitioning would provide a rationale for the different phenotypes observed in this mutant: a lower ΔpH would translate in a diminished NPQ capacity, which, along with the other modifications of photosynthesis, would lead to stress responses like the anthocyanin accumulation [25–27]. Alternatively, the higher ΔΨ [9] could enhance PSII light sensitivity, making mutant plants more stressed even at moderate light intensities.

Recently, the commonly employed procedure to deconvolute the ECS signal from other light induced spectral changes in the light, as required to evaluate the two components of the pmf, has been questioned [11]. This procedure consists in subtracting the signal measured either at one reference wavelength (around 540–550) or at two reference wavelengths (at 505 nm and 540–550 nm) from the amplitude of the ECS at its peak (around 520 nm) [13,16–24]. Therefore, we decided to re-examine ECS kinetics in the light and during the light to dark transition. We compared the “classic” procedure for the ECS deconvolution with a more detailed spectroscopic study based on the analysis of 14 different wavelengths in the 500–600 nm range in both WT and TPK3 knock-down Arabidopsis plants.

2. Methods

Plants (WT and TPK3 antisense plants, from now on TPK3 KD) were grown at 100 μmol photons m⁻² s⁻¹ for 5 weeks with a photoperiod of 16 h light/8 h dark. New mutant lines were generated following the protocol described in [16]. Mutant seeds were first germinated on MS1/2 plates containing kanamycin (50 μg/mL) for selection. Kanamycin-resistant plants were then transferred to soil and tested for post-transcriptional silencing of the TPK3 gene by mRNA extraction from 2-week-old leaves. Transcript analysis of the new clones indicated that silencing efficiency was in the range of 50–80% in the newly generated lines (not shown). Transcript analysis on leaves from plants that were used for ECS and NPQ measurements was performed as previously described [16].

In vivo spectroscopic analysis was performed with a JTS-10 spectrofluorimeter (Biologic, France) on freshly collected leaves. The detection beam was provided by a white LED source filtered through appropriate interference filters (Supplementary Fig. 1). The measure and reference photodiodes were protected from actinic light by BG 39 filters. Changes in the pmf were first evaluated from ECS changes measured at 520–545 nm [16] to disentangle this signal from the redox changes associated with the cytochrome b₆f complex turnover [28,29] and the 535 nm shift that is reflects a modification of the xanthophyll zeaxanthin during the onset (or the relaxation) of NPQ [30,31]. The amplitude of the ECS signal in the different leaves was normalized to the signal corresponding to 1 charge separation, to facilitate comparison. The latter was estimated as the amplitude of the ECS signal measured 150 μs after exposure to a saturating single turnover laser flash, under conditions where PSI is inactive (i.e. upon addition of saturating amounts of the PSI inhibitors DCMU and hydroxyamine). In these conditions, only PSI is active and therefore the ECS amplitude
corresponds 1 charge separation per photosynthetic electron transfer chain [10].

Alternatively, a more complete spectral analysis was performed, measuring the kinetics of the light to dark transition at different wavelengths from 500 to 600 nm. To this aim, the JTS-10 spectrophotometer was equipped with a rotating filter holder (JBeam Bio, France). By changing the angle between the detection light beam and the interferential filters it was possible to modify the wavelength of the detecting beam. Overall, we selected 14 different wavelengths (506 nm, 510 nm, 516 nm, 520 nm, 527 nm, 535 nm, 540 nm, 545 nm, 550 nm, 554 nm, 559 nm, 565 nm, 570 nm, 573 nm) from 7 filters centred around 507 nm, 520 nm, 527 nm, 546 nm, 554 nm, 563 nm, 573 nm). The transmission spectra of the different filters were measured with a USB 2000+ spectrophotometer (Ocean Optics, USA) directly plugged to the sample holder of the JTS-10 spectrophotometer. Spectra were measured before or after the light had crossed green or red leaves. The full width at half maximum (FWHM) was variable depending on the wavelengths (Supplementary Fig. 1), and the intensity of the light transmitted by the leaves was differentially decreased at specific wavelengths. However, the spectral characteristics of the light transmitted by green and red leaves were similar (Supplementary Fig. 1).

In the case of the experiments presented in Fig. 4, Supplementary Fig. 2 and Supplementary Fig. 3, spectra were obtained measuring the 14 wavelengths in one order and then repeating the same sequence in the reverse temporal order. Leaves were first acclimated to light for 5 min, and then kinetics of the dark relaxation were measured in the dark for every wavelength. Leaves were re-exposed to light for 2 min between two consecutive measurements. We checked that this illumination regime was sufficient to restore the amplitude of the ECS signal between two consecutive measurements. Kinetics at all wavelengths were decomposed into a sum of exponentials. The fit procedure imposes that the lifetimes values must be the same for all wavelengths, while their amplitude values are not fixed during the fit. At the end of the fitting procedure, the amplitudes corresponding to every lifetimes (i.e. the decay associated spectra, DAS) were plotted as a function of the wavelength, to evaluate the spectral features of the different components of the light to dark relaxation kinetics.

Fluorescence measurements were performed with a Speedzen II fluorescence imaging setup (JBeam Bio, France, [32]). Measuring pulses were provided by blue LEDs, while actinic light and saturating pulses were provided by red LEDs of variable intensity. NPQ was evaluated as (Fm-Fm′)/Fm′, where Fm is the maximum fluorescence emission measured in dark adapted leaves, and Fm′ is the maximum fluorescence emission measured in the light [33]. Fm and Fm′ were attained by exposing leaves to short (250 ms) saturating light pulses. Electron Transfer Rate was measured as (Fm′-F)/Fm′, where F is the fluorescence emission measured in the light, i.e. prior to exposure to the saturating pulse [33].

3. Results and discussion

Data obtained using the newly generated TPK3 KD antisense plants confirm that these plants are more prone to develop light stress signatures when exposed to moderate light (100 μmol photons m⁻² s⁻¹) than their WT counterparts. This is evidenced by the enhanced accumulation of anthocyanins in the mutant plants (Fig. 2). On the other hand, we observed some variability among the leaves of the same plant, suggesting that the generation of the ΔpH might be impaired in these leaves. To better understand this phenomenon, we decided to perform a more detailed spectroscopic analysis. Besides the ECS, other optical signal are induced in the light in the same spectral region as the ECS. These signals include, for example, the 535 nm shift that is associated to the presence of zeaxanthin during the development of NPQ [30,31] and/or the redox-induced absorption changes in the 550–555 nm region due to the turnover of the cyt b6f complex [28,29]. Since both the ECS and the 535 shift reflect a modification in the absorption spectrum of specific pigments, changes in the pigment composition could modify their relative responses. As a consequence, the overall dark relaxation kinetic could be modified, because the two signals do not necessarily have the same onset and relaxation dynamics.

To test this possibility, we re-evaluated the spectral features of the light to dark relaxation kinetic, measuring the time course at 14 wavelengths in the 500–600 nm region (Supplementary Fig. 1 and Fig. 4). We checked that despite their different pigment composition, the two types of leaves similarly transmitted the different wavelengths chosen for this spectral analysis (Supplementary Fig. 1), as required for a correct comparison. Spectral changes measured during the light to dark transition were described using a global fit procedure to determine the spectral features of the different phases of the signal relaxation. We found that four exponentials correctly reproduce the decay profile in the 0–60 s time range in “green” leaves, while only three exponentials were needed to fully reproduce the spectroscopic features of “red” leaves.

In green leaves, the first component (i.e. the fast relaxation phase in Fig. 4) has a 1/e decay time (r) of 10–20 ms, and, based on its peak suggest that the ΔpH could be diminished in the TPK3 KD mutants. Although located in the same respective positions in the rosettes of the two genotypes, mutant leaves were essentially red, while WT leaves were green in that study [16]. On the other hand, the larger heterogeneity in the leaf pigmentation observed here (with green leaves in the centre, red leaves at the periphery of the rosette in both genotypes although to different extent), allows to re-evaluate this hypothesis by extending the comparative analysis between the two genotypes to their different types of leaves.

We found (Fig. 3) that “green”, i.e. young leaves from both WT and TPK3 KD plants display similar ECS profiles. In both genotypes, the relaxation phase of the ECS was followed by a substantial recovery. According to previous interpretation of these kinetics, a substantial ΔpH is thus present in green leaves of both genotypes. On the other hand, the ECS recovery was reduced in “red” leaves, especially in the TPK3 KD plants, suggesting that the generation of the ΔpH might be impaired in these leaves. To better understand this phenomenon, we decided to perform a more detailed spectroscopic analysis. Besides the ECS, other optical signal are induced in the light in the same spectral region as the ECS. These signals include, for example, the 535 nm shift that is associated to the presence of zeaxanthin during the development of NPQ [30,31] and/or the redox-induced absorption changes in the 550–555 nm region due to the turnover of the cyt b6f complex [28,29]. Since both the ECS and the 535 shift reflect a modification in the absorption spectrum of specific pigments, changes in the pigment composition could modify their relative responses. As a consequence, the overall dark relaxation kinetic could be modified, because the two signals do not necessarily have the same onset and relaxation dynamics.

To test this possibility, we re-evaluated the spectral features of the light to dark relaxation kinetic, measuring the time course at 14 wavelengths in the 500–600 nm region (Supplementary Fig. 1 and Fig. 4). We checked that despite their different pigment composition, the two types of leaves similarly transmitted the different wavelengths chosen for this spectral analysis (Supplementary Fig. 1), as required for a correct comparison. Spectral changes measured during the light to dark transition were described using a global fit procedure to determine the spectral features of the different phases of the signal relaxation. We found that four exponentials correctly reproduce the decay profile in the 0–60 s time range in “green” leaves, while only three exponentials were needed to fully reproduce the spectroscopic features of “red” leaves.

In green leaves, the first component (i.e. the fast relaxation phase in Fig. 4) has a 1/e decay time (r) of 10–20 ms, and, based on its peak
position (around 520 nm, Fig. 5), it could represent the relaxation of the ECS signal. Therefore, this phase could reflect H⁺ flux through the ATP synthase, as proposed earlier [2,34,35]. The second phase (τ ~300 ms) also displays a peak around 520 nm, and should represent a slower decay of the ECS signal. It could reflect a slowing down of the turnover rate of the ATP synthase, which is known to occur at low values of the pmf [36]. The third phase (τ ~5 s) also displayed a main peak at 520 nm, and could therefore represent the inversion of the ECS identified previously [13]. This phase could stem from the slow equilibration of ions other than H⁺ to restore their distribution across the thylakoids in the light, due to the building of the pmf [12]. Alternatively, it could reflect a H⁺ influx into the lumen, mediated by ATP synthase complexes, which would work in the direction of ATP hydrolysis, until an equilibrium between the pmf and the phosphorylating potential (ΔG° = ΔG°′ + R*T*ln ([ADP][Pi])/[ATP])) is reached, as proposed earlier [13,37].

We found that an additional decay phase was needed in both WT

---

Fig. 3. In vivo kinetics of the relaxation of the ECS signal after illumination. Light intensity was 590 μmol photons m⁻² s⁻¹. White box: the actinic light was on; black box: the actinic light was switched off. N = 10 ± S.E.M form 4 biological replicates. See figure panels for symbols explanation.

---

Fig. 4. Time course of the light (590 μmol photons m⁻² s⁻¹) to dark relaxation measured at 14 different wavelengths in the 500–600 nm range. Datapoints (black circles) are presented along with fitting traces (surfaces). Samples (WT and TPK3 KD, green and red leaves) are indicated above the panels. N = 5 ± S.E.M. See also Supplementary Fig. 2 for a different view.
and TPK3 KD green leaves to properly reproduce the relaxation kinetic. While the τ of this phase could not be correctly resolved by our global analysis, its spectral features were once again suggestive of a change of the ECS signal. We tentatively interpret this phase as the signature of a slow relaxation of the pmf after attainment of the equilibrium with the phosphorylating potential, presumably due to a gradual cellular ATP consumption. This interpretation is in line with earlier suggestions of the existence of a "long-lasting" pmf in chloroplasts [13,37,38]. Its existence is also corroborated by the finding that NPQ relaxation in plants not only includes a fast, pH sensitive phase (qE), but also comprises a slower decay, which likely represents a slow relaxation of qE [39], due to a slow decrease of the proton gradient in the dark [40].

We note that the spectral features of the four components are not entirely identical. In particular, a contribution of cyt f redox changes could be seen (peak at ~555 nm) in the first component. Other possible contribution could be observed in the other DAS. However, due to some superposition of the measuring wavelengths (Supplementary Fig. 1), it was not possible to correctly disentangle these signals, which are probably of lower amplitude, from the ECS contribution.

Different kinetics were seen in the red leaves from both genotypes. In these samples, three exponential decays were sufficient to describe the light to dark transition kinetics (Fig. 5). The spectral features and lifetime of the first two phases were similar to those observed in green leaves, but the second phase was smaller. On the other hand, the third recovery phase (characterised by a maximum at 535 nm in green leaves) was replaced by a decay signal, which we tentatively assign to the 535 nm bandshift based on its peak position. This suggests that in these anthocyanin-rich leaves the slow dynamics of the ECS signal are modified, and therefore other spectroscopic signals (otherwise masked by the ECS) become visible.

Different hypotheses can be proposed to explain the different ECS profiles observed in green and red leaves. The changes in the ECS profile could reflect changes in the physiological state of the leaves. In particular, stress/increased age could reduce their photosynthetic performances, and affect the partitioning of the pmf. Consistent with this idea, earlier findings have pinpointed a change in the ECS partitioning in leaves where photosynthesis was decreased by lowering CO₂ availability [41]. To test this hypothesis, we measured the photosynthetic performances and light stress responses of green and red leaves by imaging chlorophyll fluorescence responses in whole plants. Indeed, one of the main signature of the TPK3 KD plants was their reduced photosynthesis and diminished NPQ capacity [16].

We found (Fig. 6) that old leaves (i.e. the ones at the outermost part of the rosette) had a decreased photosynthetic capacity, measured as a lower electron transfer rate (ETR) than their younger counterparts. This lower activity paralleled with a diminished NPQ response, likely reflecting the decreased ability to generate a ΔpH in the light.

Red leaves could also present altered expression/activity of ion channels, leading to a modification in counter ion fluxes. To test this possibility, we measured the transcript level of TPK3 in green and red leaves of both genotypes, in particular in the same leaves of 5-week old plants that were analysed also by ECS and for NPQ. We found that TPK3 KD plants expressed substantially less TPK3 with respect to WT organisms. Moreover, TPK3 expression in red leaves was reproducibly lower than in the green leaves in both genotypes (Fig. 7A, B). A comparison of the TPK3 expression levels for the four types of leaves with the ECS and NPQ values is reported in Fig. 7C and D, respectively.
4. Conclusions

Using a global spectral analysis, our study allows disentangling the ECS signal from other optical changes in the light. Thus, we can confirm that several ECS kinetic phases exist during a light to dark transition. One of these phases (phase “2” in Fig. 1) reflects an increase of the ECS in the dark, and could therefore be indicative of an equilibration of ions different from H⁺, as proposed earlier [13]. Based on the comparison of the ECS spectral and kinetic profiles (and in particular of the phase “2”) in the four types of leaves analysed in this work (green and red leaves from WT and TPK3 KD plants), several conclusions can be drawn. First, i. differences in the ECS recovery (phase “2”) are substantial between WT green and TPK3 red leaves, where the lower amplitudes of phase “2” are paralleled by diminished NPQ values. These data are in line with our previous conclusion that TPK3 participates in the regulation of plant photosynthesis [16]. On the other hand, ii. the relationship between changes in the above mentioned photosynthetic parameters and the expression levels TPK3 is complex. In both WT and TPK3 KD plants, the red leaves express significantly less TPK3 than green leaves, and this might contribute to the observed decrease of photosynthetic parameters even in WT leaves when they become red. WT and TPK3 green leaves behave similarly, although they display different expression levels of TPK3 (Fig. 7A). Moreover, TPK3 KD green leaves express lower levels of TPK3 than WT red leaves, but the ECS recovery and NPQ are larger in the TPK3 KD green leaves than in the WT red ones (Fig. 7). WT green leaves obtained from plants derived from the same batch of seeds display a slightly different expression level of TPK3 grown in two different laboratories (not shown), suggesting that growth conditions are influencing the expression of the channel. Overall, the intricate link between photosynthetic responses, light stress and the absolute TPK3 levels suggests that while this channel could modulate leaf responses via a direct role in ion equilibration across the thylakoid levels, it could play a more indirect role, by affecting photosynthesis via its consequences on plant stress, which in turn affects the pmf. Finally, iii. our previous

Fig. 6. Imaging of photosynthetic responses via chlorophyll fluorescence parameters in *Arabidopsis thaliana* plants. Top line: plant visible phenotype and false colour representation of Non-Photochemical Quenching (NPQ) values measured after 5 min of illumination in a whole rosette of a 5-weeks old plant. Brighter colours indicate higher NPQ values. Middle line: Electron Transfer Rate (ETR) measured in plants exposed to different light intensities. Bottom line: NPQ values measured in plants exposed to different light intensities. Green: kinetics measured in “green” central leaves. Blue: kinetics measured in “red” peripheral leaves. Left column: WT; right column: TPK3 KD. N = 5 ± S.E.M.
comparison of 4-week old WT leaves without anthocyan with stressed, anthocyan-rich TPK3-less leaves in the same respective position in the rosettes likely resulted in an overestimation of the difference of photosynthetic parameters among the two genotypes [16]. Indeed, the differences between WT and TPK3 KD observed under the present experimental conditions are closer to values previously reported for other genotypes (e.g. VCCN, ClC-e, KEA3 and PAM71, where changes in the range of ≤ 25% with respect to WT organisms were reported [14]).

Altogether, we propose that global ECS measurements (to properly distinguish this signal form other coexisting ones) in different genotypes, performed in parallel with expression analysis in the single examined leaves under relevant environmental conditions should provide a correct evaluation of the pmf partitioning between the proton gradient and the electric field (as proposed in reference [13]) and help assessing the role of the numerous existing channels/transporters in the regulation of the proton motive force in photosynthetic systems.

Transparency document

The Transparency document associated with this article can be found, in online version.

Acknowledgements

This work was supported by a grant from the HFSP (to G.A, L.C., I. S. and G. F.). Funds from the Labex Gral (ANR-10-Labx-49-01) to G.A and G.F. are also acknowledged. We would like to thanks Olivier Bastien (Grenoble) for help in generating the 3D plots of Fig. 4 and

Supplementary Fig. 2.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbabio.2018.07.001.

References


