ENERGY DEPENDENT NON-PHOTOSHEMICAL QUENCHING: PSBS, LHCSR AND OTHER ACTORS

Review

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Abstract. The photosynthetic apparatus of plants is capable of capturing light energy even with weak fluxes. Hence, an intense and rapid increase in irradiance should be dangerous for plants. To solve the problems caused by fluctuations of incident radiation (up to excessive), plants have developed a number of protective mechanisms, non-photochemical quenching (NPQ) of excited chlorophyll states is a key one. NPQ is conventionally considered as a set of various mechanisms that shorten the lifetime of excited chlorophyll states in a photosynthetic antenna and thereby reduce a dangerous light effect. The most rapid mechanism of NPQ is the so-called energy dependent quenching (qE) triggered by a thylakoid transmembrane proton potential. The main molecular actors of qE are xanthophylls (oxygen-containing carotenoids) and proteins of the thylakoid membrane: the antenna component LhcSR in algae and mosses and the photosystem 2 component PsbS in higher plants and some groups of green algal lineage. This review is devoted to the molecular mechanisms of qE, with the focus on PsbS-dependent quenching. The finding that PsbS does not bind pigments led to the hypothesis of PsbS-dependent indirect activation of quenching considering PsbS as a relay turning on the quenching sites in the major (LHCII) and/or minor photosynthetic antennae. Among the mechanisms of this relay, the effects of PsbS on carotenoid conformation and/or pK_a values of amino acid residues in PSII antennae are considered; the role of PsbS as a membrane lubricant or seed promoting LHCII migration within the thylakoid membrane and aggregation to ensure their quenched state is also suggested.

Keywords: *energy dependent quenching, direct quenching, indirect activation of quenching, PsbS, LhcSR, LHCII*

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INTRODUCTION

Non-photochemical quenching (NPT) of excited states of chlorophyll (Chl), discussed in this review, is an indispensable and at first sight unexpected companion of oxygenic photosynthesis. In terms the primary processes of photosynthesis, NFT is a competitor of photosynthesis for light energy. However, the presence of such a competitor is necessary for the safe organization of the entire molecular system of the photosynthetic apparatus (PSA), which is due to three circumstances: (a) the high photochemical activity of Chl in the oxygen atmosphere; (b) significant and rapid variations in light intensity at the surface of the earth even in the open space, and to an even greater extent - under the canopy of the plant community; (c) the need to carry out photosynthesis even at low light intensity reaching the plant in these conditions. Namely, knowing the molar extinction coefficient (ε) of Chl (of the order of 10^5 liters·mol^{-1} · cm$^{(-)(1)}$ [1] depending on the solvent, type of Chl and the choice of blue or red peak), it is not difficult to show, that at moderate light intensity (100 μmol photons·m$^{-(2)}$)·c^{-1}) the average frequency of absorption of light quanta by one molecule will be of the order of 1 s^{-1}, which is 2 orders of magnitude lower than the electron transfer rate along the electron-transport chain. Taking into account that such intensity approximately corresponds to natural illumination in the open space on a cloudy day, and under the forest canopy or in the water column at a depth of only a few meters can be even 10-100 times lower [2, 3], it can be estimated that at least 99.0-99.9% of the FSA time will be idle waiting for the next quantum of light, if light absorption and photochemical reaction is provided by one and the same molecule. Thus, photosynthesis under these conditions is limited by light availability, and the natural way to increase its intensity is to increase light harvesting by creating a large (containing at least 10^2 Chl molecules and/or other effective pigments) light-gathering antenna around each reaction center RC). Indeed, practically all photosynthetic organisms (unless one counts Archaebacteria, which use bacteriorhodopsin as a light-dependent generator of membrane potential) have photosynthetic antennae - pigment-protein complexes with approximately this number of pigment molecules.

However, the reverse side of the presence of photosynthetic antenna and high light collection efficiency is the vulnerability of FSA to the impact of intense light. As already mentioned, light intensity in natural conditions is characterized by significant and rapid

fluctuations - more than an order of magnitude in open spaces due to changes in cloud cover alone; under the forest canopy, changes in shading conditions and sun glare can produce $10^{(2)}$-10^3-fold fluctuations in illuminance with characteristic times of the order of minutes [2, 4]. If at some light intensity its absorption by the antenna and the subsequent reactions of electron transfer along the circuit were balanced, then at its multiple increase the antenna will saturate the electron flux, the rate of which will now become limiting, and the lifetime of excited states Chl of the antenna (Chl*) will increase, which will be no longer needed to provide energy for the photochemical reaction of charge separation in RC (Fig. 1). As a result, the probability of the singlet-triplet transition ^{1}Chl*→ ^{3}Chl* will also increase [5], and with it the probability of singlet oxygen formation in the reaction (1) with triplet Chl antenna [6-12]:

$$^3\text{Chl}^* + {}^3\text{O}_2 \rightarrow {}^1\text{Chl} + {}^{(1)}\text{O}_2. \quad (1)$$

Fig. 1. Schematic illustrating the deactivation pathways of the excited state of Chl antenna. The deactivation pathways and their effective constants for the entire pool of Chl antenna molecules are shown: via photochemical reaction (k_P, arc-shaped green arrow), radiation-free thermal dissipation (k_D, black wavy arrow), and fluorescence (k_F, vertical red arrow). The upward vertical blue arrow shows the transition of Chl to the excited state upon absorption of a quantum of light

Under such conditions of excessive light energy, a mechanism is needed to reduce the lifetime of Chl* and thus the generation of singlet oxygen. In other words, we need another "consumer" of the energy stored in the antenna. Such a consumer is always available and ready to absorb any amount of energy - it is a thermal reservoir. And it is only necessary to open a channel for thermal dissipation of energy of excited states of Chl molecules. The mechanism that opens such a channel is called "non-photochemical quenching". The word "quenching" here comes from the optical way of its observation: with the reduction of the lifetime of Chl* the intensity of its fluorescence decreases (quenches). The epithet "non-photochemical" means that this channel is unrelated to the photochemical reaction that starts a chain of electron transfer reactions. This Chl* deactivation channel, as noted at the beginning of this article, competes with energy-storing photosynthetic processes, but it is essential for protecting FSA from singlet oxygen under light-abundant conditions. A key requirement for it in such a case turns out to be its adjustability - the ability to (partially) open/close under changing light conditions and/or other conditions that result in an imbalance between light energy absorbed and light energy utilized in photosynthesis reactions. The carotenoid pigments xanthophylls (Xs) and proteins are involved in the quenching

itself and its regulation, among which, at least in higher plants, the central role is played by the S subunit of photosystem 2 (PS2), denoted PsbS, and in most groups of algae by the protein LhcSR, which is very close to the antenna complexes (in mosses, both proteins are involved in NFT).

While the role of LhcSR in NFT as a quenching center is reasonably clear, despite almost a quarter of a century of study of PsbS-dependent NFT, the mechanism of this process remains not entirely clear. Emerging new data on the induction of NFT in some mutants or model systems claim to refute the established ideas about extinguishing centers in FSA. In recent years, a large amount of of new information has become available both on macromolecular structures of supercomplexes of reaction centers and light-gathering antennas and on interactions of individual amino acid residues underlying the processes of NFT induction. This review focuses primarily on the role of PsbS in NFT. Nevertheless, for the sake of completeness, we also present here data on the functions of xanthophylls in this process.

We will preliminarily describe the pigment-protein complexes (PPCs) of the thylakoid membrane in which the quenching is realized, i.e., the antenna complexes FS2 and photosystem 1 (PS1). Since their structure differs in different photosynthetic organisms, we will focus first of all on the organization of PBCs in the "green branch" of eukaryotic photosynthetics (Viridiplantae).

CHLOROPHYLL-CONTAINING PHOTOSYNTHETIC PIGMENT-PROTEIN COMPLEXES

In the thylakoid membrane of chloroplast, Chl and carotenoids (Kar) are localized both in the photosynthetic reaction centers FS1 and FS2 and in antenna complexes. In FS2, Chl *a* molecules are contained in proteins D1 and D2 (products of *the psbA* and *psbD* genes), which form the reaction center (6 Chl *a* molecules each), and in proteins CP47 and CP43 (products of the *psbB* and *psbC* genes; contain 16 and 13 Chl a, as well as 5 and 3-4 Kar molecules, respectively [13, 14]), which form the internal antenna of FS2. The external antenna complexes associated predominantly with FS2 are formed by the products of the large *lhcb* gene family. The main antenna complex of LHCII is a homo- or heterotrimer consisting of three very similar proteins Lhcb1, -2, and -3 (homotrimer of Lhcb1 or heterotrimer of Lhcb1 with Lhcb2 and Lhcb3), each containing 8 Chl a, 6 Chl *b*, and 4 Ks: 2 lutein, neoxanthin, and violaxanthin (Vio), which can be replaced by zeaxanthin (Zea) [15]. (The green alga *Chlamydomonas reinhardtii* has a slightly larger set of homologous proteins: LhcbM1-LhcbM9 [16].) Directly at the periphery of the reaction centers of FS2, between them and the complexes of the main antennae, small monomeric antennae CP29, CP26, and CP24 (CP24 is absent in green algae) are located - products of the genes *lhcb4*, *lhcb5*, and *lhcb6*, containing 14 (11 Chl *a*+ 3 Chl *b*), 13 (10+ 3), and 11 (7+ 4) molecules and 3

Car molecules, respectively [15]. In land plants, the listed PBCs exist as supercomplexes: two FS2 complexes (denoted C), two strongly bound (S) and two moderately bound (M) main antenna complexes; such supercomplexes are denoted $C_2S_2M_{(2)}$. Under high light conditions, some of the antennae can dissociate, leaving "reduced" C_2S_2 and C_2S supercomplexes [17]. In spinach chloroplasts [18], as well as algae *C. reinhardtii* [19] and *Chlorella sorokiniana* [20], larger $C_2S_2M_{(2)}L_2$ complexes were found, including additionally two weakly bound antenna complexes (L). Thus, there are up to 200 Chl molecules per FS2 supercomplex (Fig. 2).

Fig. 2. Schematic representation of the FS2 supercomplex, $C_2S_{(2)}M_{(2)}L_2$, which includes a dimer of FS2 complexes (denoted C) and a pair each of strongly (S), moderately (M), and weakly (L) bound LHCII main antenna complexes. The supercomplex is stabilized by small antennae CP29, CP26, CP24 (the latter is present in higher plants but absent in green algae); view in the plane of the membrane (stroma side). Approximate contours of protein subunits are shown with a solid line, the location of Chl molecules without phytol "tails") is shown in green (Chl *a*) or turquoise (Chl *b*). Turquoise and pink dots show the location of iron and manganese atoms in FS2, respectively. Protein fragments intersecting in the projection on the membrane plane (in particular, the long arm of CP29 protein contacting FS2 from the stroma side) are not shown. Cryo-electron microscopy data on the structure of the supercomplex from *C. reinhardtii* (pdb code: 6KAD [16]) were used as a basis for the scheme. The putative location of the PsbS subunit is consistent with the hypothesis of Su et al. [17] for higher plants

The S subunit (PsbS) responsible for NFT induction, which is present in all groups of the "green branch" of photosynthetic organisms (Viridiplantae), is located somewhere at the border of FS2. Its exact localization is still an unsolved problem: it cannot be seen by cryo-electron microscopy, which suggests that it is either very weakly bound to FS2 or can bind to it in different positions [21]. This situation has persisted until very recently [22]. In all groups of algae and in mosses, another NFT-inducing protein, LhcSR (Light-Harvesting Complex Stress-Related protein), is also present in the thylakoid membrane. It was shown that in the moss *Physcomitrella patens* LhcSR is associated with LHCII complexes and is localized mainly in thylakoids of the stroma and slightly at the edges of the facet [23].

The second main site of Chl localization in PCA is RCs and antennae of PC1. As in the case of PC2, they form supercomplexes, and they are quite different in different groups of photosynthetic organisms. Cyanobacteria are characterized by trimers and in some cases tetramers of PC1, for which the role of light-gathering antennae is performed by water-soluble phycobilins,

but in some conditions (under iron deficiency) it can be chlorophyll-containing proteins IsiA, which have some similarity to the protein of the internal antenna of PC2, CP43, and form a ring around the trimer of PC1 [24]. Eukaryotic photosynthetics are characterized by chlorophyll-containing membrane antennae Lhcr in the red algal lineage or homologous them Lhca in the green algal and higher plant lineages. The number of such antennas per one RC of FS1 in higher plants is 4, and in green algae - 10 (it is possible that this number may change during acclimation to changes in light intensity), and antennas within each supercomplex represent different, although close proteins (Lhcr1, Lhcr2 and Lhcr3 in red algae, Lhca1-Lhca9 in green algae, Lhca1-Lhca6 in land plants). In addition, the main antenna complex of FS2, LHCII, can also dock to the antenna complexes of FS1 (Fig. 3). Apparently, it is these antennae surrounding the RCs of FS1 that prevent their tri- or tetramerization, and therefore, in eukaryotes, FS1 antenna supercomplexes contain only one FS1 complex [24]. The Chl and Kar content of a single antenna protein Lhca ranges, depending on the species and protein, from 12 to 19 molecules of Chl (9-19 Chl *a* and 1-5 Chl *b*) and 2 to 5 molecules of Kar (lutein, beta-carotene, and Vio or Zea) [25-27]. In general, it can be estimated that, taking into account 96 Chl *a* molecules in RC FS1 such supercomplexes contain up to 250 Chl *a* molecules in green algae and 150 Chl b in land plants (or approximately up to 330 and 190, respectively, in the presence of LHCII in the supercomplex).

Fig. 3. Schematic representation of the FS1 supercomplex (referred to in the literature as PSI-LHCI-LHCII), which includes the FS1 complex, several LHCI antenna complexes (products of the *lhca1-lhca9* genes), and two main antenna complexes of FS2, LHCII; view in the membrane plane. Depending on the species and light conditions, the FS1 supercomplexes may contain fewer antennae. The scheme is based on cryoelectron microscopy data on the structure of the supercomplex from *C. reinhardtii* (pdb code: 7D0J [28])

FS1 complexes are located in the stromal regions of thylakoids. As mentioned above, in organisms possessing the NFT protein LhcSR (at least in *P. patens*), it is also present in the thylakoids of the stroma and, therefore, can quench excited states of Chl in FS1 supercomplexes through interaction with LHCII antennae associated with FS1 [29]. However, the marked decrease in the role of LHCII in light harvesting for FS1 that occurred during the transition from algae to higher plants probably made LhcSR-dependent NFT unnecessary and led to the disappearance of this protein in vascular plants [30]. In addition, experimental data and results of model calculations show that under the most normal conditions (at moderately high light intensities typical of natural light), the fluorescence intensity of FS1 is relatively low, ranging from ~5 to 14% (cf. detailed

discussion in Schreiber and Klughammer [31]; FS1 fluorescence can reach 25-30%, at least in cyanobacteria and eukaryotic algae, but this occurs only at intensities of the order of 5000-8000 μmol photons m^{-2} - c^{-1} [32], i.e., at about ^{2}.5-4·5-4·0 %.i.e., about 2.5-4 times higher than the maximum possible light intensity at the Earth's surface in the subtropical zone [33]). This indicates a relatively low steady-state concentration of excited Chl states in FS1 under natural illumination conditions, which does not require the involvement of additional effective quenching mechanisms. Therefore, the quenching mechanisms discussed below are mainly related to the Chl antennas associated with FS2.

BASIC PHOTOPHYSICAL MODELS OF NON-PHOTOCHEMICAL QUENCHING

Formally, the opening of the thermal deactivation channel Chl* is described by an increase in the thermal dissipation constant k_D (Fig. 1) and is associated with the transfer of excitation to a pigment (or a group of pigments) with a short-lived excited state. Once on this trapped pigment, the excitation energy is rapidly redistributed to the thermal (vibrational) degrees of freedom of the molecule and its surroundings, which are not dangerous from the point of view of chemical activity. Such pigments or groups of pigments are called quenching centers; in fact, they really play the role of effluents for the excitation migrating along the antenna. It has been repeatedly shown that carotenoids are actively involved in the radiation-free thermal dissipation of excess energy absorbed by the photosynthetic antenna [34, 35], which is due to the short lifetime of their excited state (on the order of 10 ps [36], which is much shorter than that of Chl - on the order of 1 ns, depending on the state of FSA [37]). However, the role of a particular carotenoid in the induction of NFT can be different: it can become a quenching center itself (in this case, we speak of direct quenching) or contribute to the formation of other quenching centers, i.e., indirectly activate NFT (indirect activation of quenching).

Without dwelling here in detail on the mechanisms of direct quenching, we only note that one of the key hypotheses here was the molecular gear shift model, which considers the carotenoid Zea as the main quencher [38]. The energy estimates of the "dark" (spectrally forbidden but actively participating in energy transfer) excited state S1 of Zea and Vio made by Frank et al. [38], said that for Zea (with S1 energy lower than the energy of the first electronically excited state Chl) energy transfer from Chl to Zea followed by quenching was preferred; at the same time, for Vio (with higher S1 energy) energy transfer towards Vio was preferred→ Chl, which made it a more efficient light collector Fig. 4). This photophysical model tied in nicely with the notions of a photoprotective role for the Kar of the violaxanthin cycle, which had been shown shortly before

by Demmig-Adams [39] after three decades of obscurity of this function since the discovery of the violaxanthin cycle by Sapozhnikov et al. [40]. Later, this model was criticized: there was evidence that the S1 energies of Zea and Vio *in situ* (in LHCII) differ significantly less than *in vitro* (in solvents) [41]; that the energy transfer most likely occurs not on Zea but on lutein, at least in LHCII [42]; finally, that the magnitude (and even the sign) of the difference between the energies of Kar and Chl have little effect on the occurrence of Kar-dependent quenching [43]. As an alternative to the model of molecular transfer switching with excitation energy transfer to Zea, the model of NFT by a charge transfer (CT) mechanism has also appeared in the literature [44]. In fact, this is also Zea-dependent quenching, but due to electron transfer from Zea to Chl rather than neutral excitation energy, resulting in the formation of a rapidly recombining $Chl^{(-)}$-Zea $pair^{+}$. This mechanism also suggests a special role for Zea in NFT due to its low ionization potential (the lowest of all violaxanthin cycle carotenoids). Nowadays, the CT mechanism seems to have ceased to be considered as an essential NFT mechanism in LHCII [45], but its involvement in quenching in the small antennae of FS2, CP24, CP26, CP29 [46], and in LhcSR [47] is unquestionable.

Fig. 4. Schematic of the interaction of violaxanthin cycle pigments with Chl antennae according to the molecular gear switching model of Frank et al. [38]. The arc-shaped arrows show the transfer of neutral excitation between pigment molecules, in which the excited donor molecule switches to the ground state and the unexcited acceptor molecule to the excited state. According to the model, energy transfer between the considered pigments (Chl, Vio, and Zea) occurs predominantly in the direction of Vio→ Chl→ Zea. The wavy arrow shows the radiation-free and energy transfer-free transition of Zea* to the ground state (thermal dissipation of excitation energy).

The idea of the possibility of indirect Zea-dependent activation of NFTs in the antenna arose soon after the first model of direct quenching due to different (inconsistent with the molecular gear switching model) results of calculations of the S1 state energies of Vio and Zea [41, 48]. It was assumed that Zea could facilitate the conformational transition of LHCII to the extinguished form [41] or increase the sensitivity of LHCII to NFT-stimulated lumenal acidification [49].

We mentioned above that PsbS protein plays a central role in the regulation of NFT, along with Ks. Since the discovery of this role [50], ideas about the mechanism of its involvement in NFT have evolved from direct quenching to indirect quenching. Because of the importance of this protein for NFT, we will devote a separate section to it (as well as its ancient analog LhcSR).

INSIGHTS INTO THE ROLE OF PSBS AND LHCSR PROTEINS IN NON-PHOTOCHEMICAL QUENCHING

The PsbS protein was first isolated from spinach thylakoids in 1986 as a subunit of FS2 [51], performing an unknown function, and was initially referred to in the literature as 22-kDa polypeptide or PSII-S. Its amino acid sequence was soon established, showing its similarity to antenna proteins but, unlike them, the probable presence of four transmembrane helices in its composition [52, 53]. In 1995, it was reported - as it later became clear, erroneously - that this protein binds 5 molecules of Chl *a* and *b* and several types of carotenoids; therefore, it was suggested that its function was related to additional and/or alternative light harvesting [54]. This error subsequently had a major impact on the ideas about its mechanism of function. Five years later, studies of *Arabidopsis thaliana* mutants obtained by nondirected mutagenesis (irradiation with fast neutrons) revealed that this protein is key for NFT [50]. Later it was shown that, in addition to suppression of NFT and increased sensitivity of FSA to photoinduced damage in the absence of PsbS, NFT and protection against FSA damage by excessive light are more pronounced in its overexpression than in the wild type [55, 56]. As was already known by the late 2000s, many organisms belonging to the green lineage (Viridiplantae) have putative PsbS orthologs of higher plants in their genome. Despite this, the PsbS protein was not initially detected either in the classical model green alga *C. reinhardtii* or in any of the other species of microscopic green algae studied (for current views on the distribution of this protein among photosynthetic organisms, see "Distribution of non-photochemical quenching mechanisms in different systematic (evolutionary) groups" of this review). Oddly enough, however, it was found in the multicellular green alga *Ulva laetevirens*, while its presence was extremely minor in the char alga *Chara corallina* [57]. The fact that, nevertheless, some unicellular species demonstrated high NFT activity made us assume that some mechanism independent of PsbS was at work in them. Indeed, the LhcSR protein (Light-Harvesting Complex Stress-Related protein, previously described as LI818 [58]), responsible for NFT and distributed (if we speak about orthologs of its gene) in all eukaryotic algae except red algae (Rhodophyta), as well as in mosses, was soon discovered in *C. reinhardtii* [59].

Both proteins, LhcSR and PsbS, belong to the large superfamily of LHC - antenna proteins that bind Chl and Ks [30]. The LhcSR protein has a spatial structure similar to that of the small antenna of FS2 (CP29) or the monomers of the LHCII complex (i.e., a structure including three transmembrane alpha-helical columns; Fig. 5), and, like them, also binds Chl and Cs molecules: 8 Chl molecules (8 Chl *a* in the LhcSR1 isoform, 7 Chl a and 1 Chl *b* in LhcSR3) and 3 or 4 Cs molecules, including lutein and Vio [47, 60]. There are different ideas about how LhcSR (and

closely related LHCX proteins) causes quenching of Chl electronic excitation in the antenna: through excitation transfer from Chl to followed by rapid conversion of Cc* to Cc; through electron transfer from Cc to Chl; or through the formation of exciton coupling between Chl and Cc molecules (we discussed above various putative mechanisms of direct Kar-dependent quenching), but in any of these cases it is assumed that it is LhcSR that is the center of quenching [47, 61].

Fig. 5. Structure of the S subunit of FS2, PsbS (*a*), by X-ray structural analysis (pdb code: 4RI2 [62]), and the monomer of the LHCII main antenna complex, Lhcb1 (*b*), by cryo-electron microscopy (pdb code: 8IX0 [63]); the sequence of the proteins constituting LHCII is close to that of LhcSR, for which an experimental three-dimensional structure has not yet been obtained to date. The helical regions (α and 3_{10}) are shown as cylinders and are signed for both proteins (TM1-TM4 are transmembrane helices, H1-H3 are PsbS amphiphilic helices; in Lhcb1, their common designations are A-C and E-D, respectively). Chl molecules bound to Lhcb1 are highlighted in green (Chl *a*) or turquoise Chl *b*; phytol "tails" are shown by a thin line), carotenoids - in yellow (lutein), orange (neoxanthin), and red (violaxanthin).

For some time, a similar mechanism of direct quenching was assumed for PsbS [50, 64]. However, data on the biochemical properties of PsbS [65], as well as data on PsbS mutagenesis [57], and then data on the three-dimensional structure of this protein [62] showed that, contrary to the first reports [50, 54], it does not contain pigment molecules. Unlike LhcSR and antenna proteins, PsbS has an additional (fourth) transmembrane helix (Fig. 5) and a more compact structure that does not leave sufficient space for Chl and Ks binding [62]. It is interesting to note that the three-dimensional structure of PsbS is quite conservative despite the rather high variability of the amino acid sequence of PsbS (for example, the proteins of spinach and the green alga *Lobosphaera incisa* share only about one third of common residues [66]).

In this connection, various hypotheses were put forward about a possible indirect mechanism of PsbS participation in the quenching of excitation in the antenna, realized through its interaction (directly or with the participation of Ks molecules) with proteins of the external and internal antennae and switching these antennae themselves to the "quenching" state.

Note, however, that regardless of the molecular mechanisms by which LhcSR- and PsbS-dependent quenching is ensured, it is believed that each of these proteins serves as a pH detector in the thylakoid lumen and, therefore, provides an energy-dependent component of NFT (qE; in this review, we use this generally accepted designation not for the physical quantity - a measure

of the quenched fluorescence by means of the energy-dependent quenching mechanism, but for this mechanism itself) [30]. Acidification of lumen leads to protonation of amino acid residues exposed in lumen and formation of a quenching center in the protein itself or in the antenna complexes in contact with it.

PUTATIVE MECHANISMS AND CENTER OF PSBS-DEPENDENT NON-PHOTOCHEMICAL QUENCHING

The absence of pigments in PsbS means that it cannot serve as an extinguishing center, but can only stimulate the formation of extinguishing centers in neighboring PBCs. There is experimental evidence that, at least in higher plants, the extinguishing center is formed within the trimer of the LHCII main antenna [67-69]. Although the unextinguished (light-gathering) conformation of LHCII is thermodynamically somewhat more favorable at room temperature than the extinguished one, the free energy of the transition between them is very small: only about 2.5 *kT* (*k* is Bolman's constant; *T* is absolute temperature) , i.e., close to the characteristic energy of thermal motion [70]. Apparently, therefore, the switching of LHCII into the quenched state (i.e., the formation of a quenching center in it) can occur under the action of a variety of relatively "weak" stimuli [71]. These stimuli include: changes in thylakoid membrane stiffness [72, 73]; thinning of the thylakoid membrane [74]; the occurrence of transmembrane asymmetry - accumulation of digalactosyl-diacylglycerol (DGDG) on the lumen side [74]; aggregation of LHCII 75, 76]; and the presence of Zea [77] or PsbS [78-81]. However, it is not entirely clear which of these causes are primary. Under physiological conditions, the primary reason for the development of quenching in the antenna is thylakoid transmembraneas a necessary and sufficient condition for qE, whereas Zea and PsbS with additive effects [80] can regulate the sensitivity of qE to. At the same time, full-fledged qE can be obtained in LHCII by increasing ΔpH to unphysiologically high values (more than 3 units) even in the absence of Zea or PsbS [68, 80] and in the absence of small antennas [68].

The direct mechanism for the induction of quenching is thought to be related to the convergence of the LHCII-bound pigments, Chl a 612 and lutein -620 (its binding site in the protein is also designated Lut1; Fig. 6), which enhances their exciton interaction [82-84]. Indeed, according to molecular modeling data, the exciton coupling between Chl *a* 612 and lutein-620 changes from 5-9 cm^{-1} (distribution maximum) to 12-15 cm^{-1} upon acidification [71]. Their convergence, in turn, may be caused by neutralization (due to protonation) of the amphipathic (amphipathic) helix D, parallel to the membrane, allowing it to position itself closer to the

hydrophobic core of LHCII [85]. Another putative variant of the conformational rearrangements responsible for the transition of LHCII to the silenced state is a change in the angle between transmembrane helices A and B [83, 84]. Finally, it is also possible that due to lumen acidification and protonation of Glu-94, its hydrogen bonding partner changes from Lys-99 to Gln-103 (Fig. 6) [84], leading to a decrease in the average distance in LHCII between helices E and D.

Fig. 6. Structure of Lhcb1, as measured by cryo-electron microscopy (pdb code: 8IX0 [63]); only the chlorophyll cluster Chl *a* 610, -611, -612, and lutein (in the Lut1 binding site), presumably forming the quenching center, is shown. The side chains of Glu-94, Lys-99, and Gln-103, presumably associated with the transition of LHCII to the quenched state, are shown in color. For other explanations, see the caption of Fig. 5

Protein-lipid interactions and the local lipid composition of the membrane near LHCII play a role in these conformational changes. Thus, it has been shown that at neutral pH the LHCII trimer is surrounded by a large amount of the membrane galactolipid monogalactosyl-diacylglycerol (MGDG) [86], which has a conical shape and is not characteristic of flat bilayer membranes. At low lumenal pH, the cylindrical-shaped lipid DGDG can accumulate in the thylakoid membrane, in its lumenal sheet near LHCII. Molecular modeling shows that this is due to the compactization of LHCII on the lumenal side and the fact that DGDG has a better steric match in this case and fills the cavities formed [74]. However, it reduces the mobility of LHCII, thereby preventing its complexation, which is necessary for the induction of NFT [69]. It is assumed that PsbS, by binding to LHCII and displacing DGDG, restores the mobility of LHCII in the membrane. At the same time, PsbS itself also changes its conformation upon lumen acidification to bind to LHCII [87, 88]. In other words, PsbS may play the role of a "membrane lubricant" [74, 89, 90]. This is also supported by experimental data: the absence of PsbS (in the *npq4* mutant) promotes the formation of ordered (crystalline) phases in which proteins are immobilized [81]. This function of PsbS possibly involves Zea, which ensures efficient binding between LHCII and PsbS in the photoprotective state [87, 90, 91]. At the same time, unbound Zea can induce changes in thylakoid membrane stiffness, which alters the lateral pressure in the membrane; as a result, protein-lipid interactions are altered and conformational changes in LHCII are also induced, putting it in a dissipative state [72, 73]. In the absence of Zea, qE activation requires a decrease in lumenal pH to 4.7-5.0 [49].

In addition to its effect on LHCII aggregation, it is hypothesized that PsbS may also regulate the pK_a values of lumen-exposed LHCII residues as well as other antenna proteins (e.g.,

the minor antenna CP29, by interacting with which PsbS can increase the pK_a value of the Glu-128 residue from 4.46 to 5.24 [92]). It is possible that Zea, the most hydrophobic xanthophyll, may serve a similar function by being located both within and at the periphery of LHCII; it may thereby increase the pH sensitivity of LHCII, allowing its protonation and induction of qE at moderate ΔpH [49]. Finally, it has been suggested that PsbS induces quenching by changing the conformations of carotenoids in LHCII, as it has been shown that these changes may be responsible for switching between light harvesting and photoprotection modes [93].

However, whatever the mechanism of PsbS-dependent activation of NFT, it must be turned on when the pH in the thylakoid lumen is lowered. After it was discovered that PsbS can exist in the thylakoid membrane in both monomer and dimer forms (and the equilibrium between the monomer and dimer forms reversibly changes in a light-induced manner, shifting toward the monomer form when is lowered), the hypothesis that the pH-dependent activation of PsbS is due to its conversion from a dimer to a monomer is considered as one of the main hypotheses [94]. It has been suggested that this monomerization is accompanied by a change in the location of PsbS within the FS2 supercomplex, namely the association of monomers with light-gathering antennae, while dimers tend to associate with the core of FS2. It has recently been suggested that the dimer↔ monomer transitions are in turn associated with changes in the two short amphiphilic helices H2 and H3 on the lumenal side of the protein. For example, NMR and IR spectroscopy data revealed a relocation of the H2 helix in the PsbS protein of the moss *P. patens* from the aqueous phase to the membrane phase and a transition of H3 from the loop segment to the helix caused by protonation of key glutamate residues [95]. At the same time, the X-ray diffraction study of PsbS showed that the active form of PsbS is also a dimer [62].

A schematic summarizing the basic concepts of PsbS- and LhcSR-dependent quenching is shown in Fig. 7.

Fig. 7. Schematic representation of the basic ideas about the mechanism of PsbS- (*a*) and LhcSR-dependent quenching (*b*). Schematically shown are images of the supercomplexes FS2 and FS1 (in projection on the membrane plane) formed by the complexes of one of the reaction centers (FS2 or FS1) and the surrounding antenna complexes of the main light-gathering antennae, LHCII and/or LHCI, and small antennae (CP29, CP26, CP24). The images on different color backgrounds correspond to different characteristic pH values. It is emphasized that under slightly acidic conditions (pH< 6) PsbS activates NFTs in antenna complexes by directly interacting with them, or by stimulating their separation from FS2 supercomplexes and subsequent aggregation. At the same time, significant acidification of the medium (pH< 4) induces the formation of quenching centers in the main antenna complexes of LHCII even in the absence of PsbS. Panel (*b*) reflects

the notion that LhcSR itself forms a quenching center (energy sink) for excitation in the LHCII main antenna regardless of whether it is associated with FS2 or FS1. The schematic at the top of panel (*a*) illustrates the putative mechanisms of indirect PsbS-dependent activation of NFTs in LHCII and small antennae. The depiction of supercomplexes of reaction centers and antennae does not reflect the difference between their structure in algae and land plants, the specific location of PsbS and LhcSR is shown conventionally, the designation of the quenching center does not indicate its localization within individual antenna PBCs or their aggregates

NON-PHOTOCHEMICAL QUENCHING AS REGULATION OF UNIVERSAL FLUORESCENCE FLUCTUATION

It is still difficult to say whether all the described mechanisms of NFT and to what extent they are realized *in vivo*, but the diversity and complexity of molecular mechanisms of NFT regulation are undoubted. The reasons for this diversity and the origin of NFTs may be elucidated by studies of fluorescence intermittency (fluorescence intermittency or blinking). This universal phenomenon was discovered in the for a wide class of fluorescent systems: from organic pigments to quantum dots [96, 97].

The introduction of single molecule spectroscopy into spectral studies allowed us to discover a phenomenon masked by ensemble averaging: stochastic switching of the molecular fluorescent system between bright and dim states, i.e., states with different quantum yields of fluorescence. The transitions between these states are thought to be related to the fluctuating microenvironment of the fluorophore. This flickering was also detected in photosynthetic protein complexes - in the main photosynthetic antennae of FS2 of LHCII plants [98], in the antennae of purple LH-2 bacteria [99], in allophycocyanins of cyanobacteria [100], and in B-phycoerythrin of red algae [101]. Studies of the main antenna complex of higher plants LHCII have shown that, unlike simple systems, in such complexes with dense packing of a large number of pigments (8 Chl *a*, 6 Chl *b*, and 4 Kar) a number of states with different levels of "luminosity" arise [98]. The transitions between these states occur spontaneously, and the lifetime of LHCII in each of them ranges from tens of milliseconds to tens of seconds (although ***most*** of the times lie in the second and sub-second ranges). At the same time, both the frequency of transitions and the lifetime in each of the states depend on the environmental conditions: the pH value and the intensity of the excitation light [98]. Thus, both the light-gathering and the "attenuated" states of the antenna are inherent to this and many other PBCs and do not require any special modifications of the protein (by binding additional pigments or protonation of amino acid residues); the latter are necessary

only to stabilize one of these states. In other words, we can say that the plant does not "construct" a new channel of excitation quenching in the antenna, but uses intrinsic states with different levels of quenching and only creates conditions for shifting the dynamic equilibrium toward the one that is necessary under the given physiological conditions. The universality of the phenomenon of fluorescence fluctuations, far from being limited to photosynthetic antennas, but underlying the regulation of light harvesting processes in photosynthesis, creates the basis for understanding the evolution of these adaptive processes.

DISTRIBUTION OF NON-PHOTOCHEMICAL QUENCHING MECHANISMS IN DIFFERENT SYSTEMATIC (EVOLUTIONARY) GROUPS

The presence of multiple regulatory and, in particular, photoprotective mechanisms is characteristic of all oxygenic phototrophic organisms, and many of them are universal (i.e., characteristic of all or almost all phototrophs). At the same time, when comparing large evolutionary groups among themselves, one can see that there is also a certain specificity in the choice of mechanisms by representatives of different groups of organisms. Above we spoke about the antenna-like proteins LhcSR and PsbS responsible for quenching: direct NFT in the case of LhcSR and indirect in the case of PsbS. However, the distribution of these proteins and hence of LhcSR- and PsbS-dependent quenching is significantly different. LhcSR is common among algae (in most organisms belonging to the red algal lineage, the homologous LhcSR proteins are designated LHCX) and is also found in mosses but is lost in higher plants. On the contrary, PsbS is a characteristic protein of terrestrial (higher) plants and the most closely related characeous algae, but is absent in most other groups of algae [30, 102, 103]. It was recently believed that although the gene for this protein was present in the model green alga (Chlorophyta) *C. reinhardtii*, the protein itself was not expressed or at least not accumulated in it [102]. Only in 2016two groups simultaneously detected in *C. reinhardtii* both stress-induced differential expression of the PsbS-encoding genes *psbs1* and *psbs2* [104] and the protein itself [104, 105]. Later, temporal formation of this protein and induction of its expression were found during diurnal changes in the light intensity of *C. reinhardtii* culture [106]. However, in all cases, in contrast to LhcSR, the appearance of PsbS and its transcripts was relatively short-lived (hours), which led researchers to conclude that PsbS has only an auxiliary function and not a very significant role in quenching in this organism [104, 105]. Insignificant diurnal fluctuations in the expression of LhcSR- and PsbS-encoding genes corresponding to diurnal fluctuations in temperature and illumination were also found in *Ulva prolifera* [107].

Finally, we recently detected a long-term (persisting for several days) stress-induced differential expression of PsbS-coding gene in the green microalga *L. incisa* [108]. Such a prolonged and significant increase in the number of transcripts of this protein (by 3-4 orders of magnitude) may indicate its essential and independent role in the induction of NFT at least in some representatives of Chlorophyta. All these recent data significantly expand the distribution of PsbS among oxygenic phototrophs and extend the history of its photoprotective function in the plant world.

It should also be noted that there was recently a report on the detection of differential expression of PsbS in the red alga *Kappaphycus alvarezii* [109]. The coding sequence of the gene, kindly provided to us by the authors, reveals marked similarity to the *psbs* sequences of green algae. However, we have not yet been able to detect a homologous gene in other red algae. According to the current understanding, PsbS is present only in the "green lineage" of plants (Viridiplantae) [103], while in red algae, whose antennae contain phycobilin but not Chl [110], the function of PsbS as an NFT inducer is meaningless. If in the future the presence of *psbs* homologs in various representatives of red algae (Rhodophyta) is confirmed, it will be a turning point in the development of our ideas about the evolution of the function of this protein.

Despite the different mechanisms by which LhcSR and PsbS induce fluorescence quenching, in both cases the signal for NFT induction is the product of light reactions - acidification of the thylakoid lumen. In cyanobacteria (in a significant part of them, although not in all), which are characterized by a different system of light harvesting using unfolded tetrapyrrole pigments localized in large soluble protein complexes - phycobilisomes - a different molecular system of NFT is used. The signal for its trigger is directly high illumination, and the main participant is orange carotenoid protein (OCP) and its associated ketocarotenoid (echinenone, hydroxyecchinenone, or canthaxanthin) [111]. In this case, the level of sensitivity of the trigger mechanism to light intensity is determined by the very low quantum yield of photoconversion of OCP to the photoactive form; as a result, photoconversion occurs only at high radiation intensity [112]. The OCP-dependent NFT mechanism is characteristic even of the most primitive of the currently existing group of cyanobacteria, Gloeobacteria, which retains many ancestral characteristics [111]. Surprisingly, this mechanism is not preserved in red algae, which also possess phycobilisomes (no ocp-like genes were found in them) [113], and pH-dependent NFT mechanisms operate [114, 115].

CONCLUSION

NFT is one of the main mechanisms for protecting the PCA from dangerous excess light energy. We have considered here only one of its components, the so-called energy-dependent quenching (qE), and only one of its aspects, the role of the photoprotective subunit of PS2, the PsbS protein. Despite the intensive research in this field during all 25 years since the discovery of the NFT-inducing effect of PsbS [50] and the abundance of new data, it seems still difficult to integrate them into an overall consistent picture. For example, the mechanism of indirect PsbS-dependent activation of NFTs - through effects on $pK_{(a)\ of}$ lumenal protonated residues in LHCII and/or small antennae [92], through changes in the conformation of associated pigments [93] or through changes in LHCII mobility [74] - remains hypothetical and requires a way to reconcile it with the recently formulated model of quenching regulated by changes in environment polarity [45]. Despite the seemingly long and reliably demonstrated role of monomeric small antennae in NFTs as quenching centers [46, 116] interacting with PsbS [117], recent data on NFTs in strains of *A. thaliana* deficient in these antennae appear to warrant the conclusion that they play no role in qE [68]. It is possible that the presence of such mutually exclusive data on NFT mechanisms indicates that all of them can be realized to some extent *in vivo*; the system as a whole has a sufficiently high "buffer capacity" of its defense mechanisms so that disabling one of them in a model system would not significantly affect the overall level of NFT.

Finally, the paradoxical fact that PsbS, a representative of the superfamily of antenna (i.e., chlorophyll-binding) proteins that lacks any pigments and, together with them, the ability to directly extinguish, is the main participant of the NFT. It can be assumed that this is another candidate for the collection of examples of imperfect evolutionary solutions based on the use of "improvised" material and associated with the change of functions.

Photophysical mechanisms of quenching related to the interaction of pigments (Chl and Kar) on picosecond time scales have remained beyond detailed consideration. Despite the long history of research on these mechanisms, a consensus in their understanding has not yet been reached either. We have left aside other photoprotective mechanisms that also reduce the lifetime of the excited state of Chl in the photosynthetic antenna and, therefore, can be fully attributed to NFTs. This is primarily the mechanism that modulates the intensity of qE by synthesizing an additional amount of Zea and is denoted qZ [118]. Key to this mechanism are the enzymes violaxanthin-deepoxidase and zeaxanthin-epoxidase, which carry out the violaxanthin cycle and are responsible for the quasi-stationary concentration of Zea [119]. It is also a mechanism that has not yet received a satisfactory name in the Russian-language literature and is known as "state transition" (qT). The trigger for this mechanism is not transmembrane ΔpH, but rather the redox

of the plastoquinone pool on the acceptor side of FS2 [120], which activates the kinase that phosphorylates LHCII complexes. As a result, they lose affinity for FS2 and migrate to a less hydrophobic region rich in FS1 complexes [121], in contact with which they find an efficient efflux of absorbed light energy. It can be assumed that the diversity of these defense mechanisms, as well as the diversity of possible ways of realization of energy-dependent quenching alone, indicates the importance of this function and the desire of the photosynthetic organism to use any mechanism of protection of PCA, which turned out to be sufficiently effective.

AUTHORS' CONTRIBUTIONS

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest in financial or any other field.

ETHICS DECLARATION

This article does not describe any studies performed by the authors involving humans or using animals as subjects.

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