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REVIEW

Chlorophyll *a* fluorescence induction: a personal perspective of the thermal phase, the J–I–P rise

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Abstract The fast (up to 1 s) chlorophyll (Chl) *a* fluorescence induction (FI) curve, measured under saturating continuous light, has a photochemical phase, the O–J rise, related mainly to the reduction of Q_A, the primary electron acceptor plastoquinone of Photosystem II (PSII); here, the fluorescence rise depends strongly on the number of photons absorbed. This is followed by a thermal phase, the J–I–P rise, which disappears at subfreezing temperatures. According to the mainstream interpretation of the fast FI, the variable fluorescence originates from PSII antenna, and the oxidized Q_A is the most important quencher influencing the O–J–I–P curve. As the reaction centers of PSII are gradually closed by the photochemical reduction of Q_A, Chl fluorescence, *F*, rises from the O level (the minimal level) to the P level (the peak); yet, the relationship between *F* and $[Q_A^-]$ is not linear, due to the presence of other quenchers and modifiers. Several alternative theories have been proposed, which give different interpretations of the O–J–I–P transient. The main idea in these alternative theories is that in saturating light, Q_A is almost completely reduced already at the end of the photochemical phase O–J, but the fluorescence yield is lower than its maximum value due to the presence of either a second quencher besides Q_A, or there is an another process quenching the fluorescence; in the second quencher hypothesis, this quencher is consumed (or the process of quenching the fluorescence is reversed) during the thermal phase J–I–P. In this review, we discuss these theories. Based on our critical examination, that includes pros and cons of each theory, as well mathematical modeling, we conclude that the mainstream interpretation of the O–J–I–P transient is the most credible one, as none of the alternative ideas provide adequate explanation or experimental proof for the almost complete reduction of Q_A at the end of the O–J phase, and for the origin of the fluorescence rise during the thermal phase. However, we suggest that some of the factors influencing the fluorescence yield that have been proposed in these newer theories, as e.g., the membrane potential $\Delta\Psi$, as suggested by Vredenberg and his associates, can potentially contribute to modulate the O–J–I–P transient in parallel with the reduction of Q_A, through changes at the PSII antenna and/or at the reaction center, or, possibly, through the control of the oxidation–reduction of the PQ-pool, including proton transfer into the lumen, as suggested by Rubin and his associates. We present in this review our personal perspective mainly on our understanding of the thermal phase, the J–I–P rise during Chl *a* FI in plants and algae.

Keywords Bioenergetics · Chlorophyll *a* fluorescence · Fluorescence induction · OJIP transient · Mathematical modeling · Photosynthesis · Thermal phase

This perspective/review is written in honor of C. Barry Osmond for his extensive contributions to photosynthesis research in plants; he is a pioneer of plant physiology, photoprotection by xanthophyll pigments, photoinhibition, and photosynthetic efficiency of plants under varied ecological conditions.

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"If we study the history of science we see produced two phenomena which are, so to speak the inverse of the other. Sometimes it is simplicity which is hidden under what is apparently complex; sometimes, on the contrary, it is simplicity which is apparent, and which conceals complex realities"—Henri Poincare (1854—1912); see Science and Hypothesis, Dover Publications, New York, 1952

Introduction

Chlorophyll (Chl) *a* is 'the chosen' pigment molecule for oxygenic photosynthesis, as it is the only member of the Chl family present in all organisms that carry out oxygenic photosynthesis, from primitive cyanobacterial cells to sequoia trees (Bjorn et al. 2009); the only possible exception is Chl *d* in *Acaryochloris* (Ohashi et al. 2008; Allakhverdiev et al. 2011). Depending on its protein environment, Chl *a* functions either as a light harvester, or as a redox participant in the primary charge separation in the reaction centers (RCs) of Photosystems II (PSII) and Photosystem I (PSI) (Clegg et al. 2010; Renger 2010, 2011). A small part of the light energy absorbed by Chl *a* is dissipated as heat (internal conversion), and as fluorescence ($\sim$2–10 %, Latimer et al. 1956, 1957; Trissl et al. 1993). The emission spectrum of Chl *a* fluorescence, at room temperature, is characterized by a major peak centered around 685 nm (attributed mainly to light-harvesting antenna in PSII) and a broad shoulder between 700 and 750 nm (that includes the vibrational sub-band of PSII Chl *a* emission and an emission band from PSI Chls). PSI contribution to the overall fluorescence signal at room temperature is $\sim$5–30 % for C3 plants (Lavorel 1962; Wong and Govindjee 1979; Roelofs et al. 1992; Pfündel 1998; Gilmore et al. 2000; Rappaport et al. 2007). PSI fluorescence is practically constant during illumination (Byrdin et al. 2000; Govindjee 2004). However, the fluorescence emitted by PSII varies with time when the photosynthetic samples, which had been kept in darkness, are illuminated; Chl *a* fluorescence yield change has fast (up to $\sim$1 s) and slow (up to a few min) phases. This is called fluorescence induction (FI), fluorescence transient, or simply the Kautsky effect (Kautsky and Hirsch 1931; see also www.fluoromatics.com/kautsky_effect.php; Govindjee and Papageorgiou 1971).

The intimate connection of several essential photosynthetic processes with Chl *a* fluorescence makes it a very important resource of information, particularly for the structure and function of PSII; it is 'a signature of photosynthesis', which is especially true when various aspects of fluorescence (e.g., kinetics; spectra; lifetimes) are exploited (Papageorgiou and Govindjee 2004; Baker 2008). Figure 1 shows all the four major protein complexes, including PSII, that are located on the photosynthetic membrane; the figure legend provides the names of all the pertinent intermediates and other details needed to understand the discussion in this review.

For higher plants and algae, the FI curve measured under continuous light has a fast (within a second) increasing phase (from a minimum F_0, to a maximum F_P), and a slow (within a few minutes) decreasing phase (toward a terminal steady state level F_T); the FI curve has several inflection points (Fig. 2a, b; see Govindjee 1995 for a history of the nomenclature used for fluorescence transient curves). The earliest nomenclature was the OPS transient (O for "origin", P for peak, and S for steady state; Lavorel 1959). Years later, the fast phase was labeled as OIDPS, where I was an inflection, and D was a dip (Munday and Govindjee 1969a, b); and then it was labeled as OI_1I_2PS (Schreiber 1986; Neubauer and Schreiber 1987), where I_1 and I_2 were two intermediate inflections. We will, however, use the alternative notation, J and I, which was introduced by Strasser and Govindjee (1991, 1992), where J and I are the intermediate inflections (Fig. 2a). The slow phase was called PSMT (Papageorgiou and Govindjee 1968a, b), where S stands for semi-steady state, M for a maximum, and T for a terminal steady state level (Fig. 2a, b); sometimes the maximum M is missing, or several semi-steady states and maxima, labeled as S_1M_1 and S_2M_2, are observed (Govindjee and Satoh 1986), which are caused by temporary limitations at the electron acceptor end of the PSI.

In this review we will discuss only the fast FI in higher plants and algae, focusing particularly on the thermal phase over the photochemical phase of the O to P rise (see Morin 1964; Delosme 1967).

It is important to note that the techniques used to measure FI in various photosynthetic samples have evolved, especially in the last 30 years, due to the development of photometric and electronic detection technologies, as well as of computer-assisted system analysis (Rottgers 2007). Instruments that have been used in FI studies include: (1) Plant Efficiency Analyser (PEA) fluorometer (www.hansatech-instruments.com; Strasser and Govindjee 1991, 1992; Strasser et al. 1995, 2000, 2004; Stirbet and Govindjee 2011), which provides a low noise data acquisition with 10 µs time resolution; (2) Pulse-Amplitude-Modulation (PAM) fluorometer (www.walz.com, or www.optisci.com; Schreiber 1986, 2004), which has a time resolution >10 µs, and is commonly used in saturating pulse (SP)-mode for the determination of fluorescence parameters associated with the slow FI (including the measure of the recovery of the initial fluorescence yield after illumination pulses); (3) Fast-Repetition-Rate (FRR) fluorometry, which is a multiple excitation technique using short saturating flashes (STFs) (Mauzerall 1976); modified and extended versions of FRR technique, with time resolution as fast as 1 µs, have been developed by Kolber et al. (1998) and Nedbal et al. (1999) (see http://www.psi.cz).

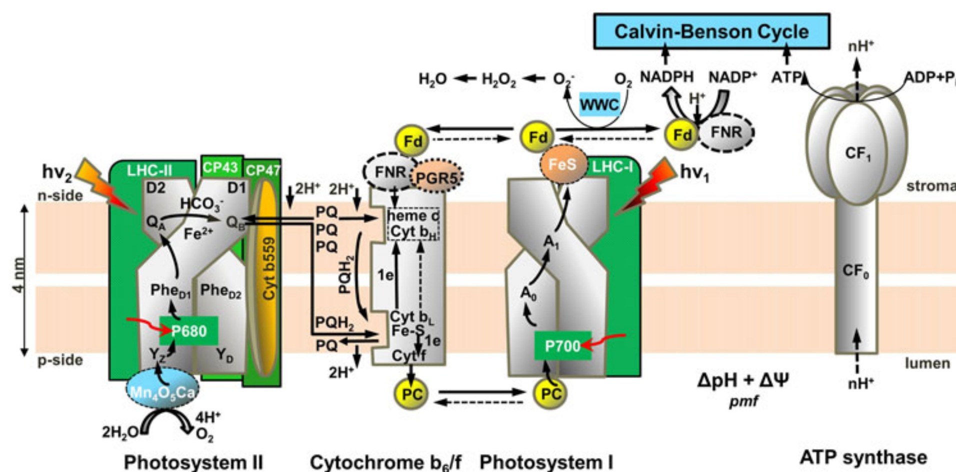


Fig. 1 A schematic representation of the four major protein complexes in the thylakoid membranes of oxygenic photosynthesizers. The major intramembranous components are: the two photosystems (PSI and PSII), connected in series via the cytochrome (Cyt) b_6/f complex, and the enzyme ATP synthase. Photosynthesis begins with the simultaneous absorption of light energy (shown with *wavy arrows*) by the light harvesting complexes (LHC) and other antenna subunits associated with PSI and PSII. In the PSII, charge separation takes place within the D1 protein of the PSII core complex, leading to the formation of the radical pair $[P680^+Phe_{D1}^-]$, where P680 (that involves the participation of several Chl *a* molecules in PSII core complex, not shown) and Phe_{D1} (D1-pheophytin) are often denoted as primary electron donor and acceptor, respectively. The second pheophytin molecule, Phe_{D2}, in principle, does not participate in primary reactions. Afterwards, $P680^+$ receives, via Y_Z (D1-Tyr161), an electron, ultimately extracted from specific water molecules by the tetranuclear manganese–oxygen–calcium cluster (Mn_4O_5Ca) of the oxygen-evolving complex (OEC). Y_D (D2-Tyr160) is an electron donor also, but it is a very slow donor. On the electron acceptor side of PSII, Phe_{D1}^- transfers the electron to Q_A (a one-electron acceptor plastoquinone, tightly bound on a site on the D2 protein), and then to Q_B (a two-electron acceptor plastoquinone, on the D1 protein) that binds weakly, but tightly when reduced to Q_B^-. A bicarbonate ion (HCO_3^-) is bound to a non-heme iron (Fe^{2+}) that sits between Q_A and Q_B; it is known to be involved in Q_B^{2-} protonation reaction. PQ and PQH_2 in the scheme refer to plastoquinone and plastoquinol molecules, respectively, of a mobile PQ-pool in the thylakoid membrane. Cyt b_6/f complex contains the following intersystem components of the electron transport chain: an iron-sulfur (Fe–S) protein, known as Rieske FeS protein, a cytochrome f (Cyt f), two cytochrome b_6 (i.e., a low potential, Cyt b_L (also called b_p) and a high potential, Cyt b_H, also called b_n), and a heme c. At the Cyt b_6/f, PQH_2 is re-oxidized at a site close to the electrochemically positive side of the membrane (p-side; toward the lumen), and PQ is reduced at a site close to the electrochemically negative side of the membrane (n-side: toward the stroma). Associated with Cyt b_6/f complex there is also a ferredoxin-$NADP^+$-reductase (FNR), and the so-called proton gradient regulator (PGR5), involved in the cyclic electron transport around PSI (CET-PSI) via ferredoxin (Fd). In PSI, primary charge separation leads to the formation of the radical pair $[P700^+A_0^-]$, where P700 (a special Chl *a* pair) is the primary electron donor, and A_0 (a special Chl *a* molecule) is the primary electron acceptor. Then, $P700^+$ is reduced by plastocyanin (PC), a mobile water-soluble copper protein, situated on the lumen side of the membrane, which transfers electrons from Cyt f to PSI (there is more than one PC molecule per PSI). On the electron acceptor side of PSI, there are: A_1 (vitamin K1); three iron–sulfur centers (shown as FeS); and the mobile water-soluble ferredoxin (Fd), situated on the stroma side of the membrane (there is more than one Fd molecule per PSI). The reduced Fd can transfer the electron to: (1) $NADP^+$ (nicotinamide–adenine dinucleotide phosphate), which is reduced to NADPH via FNR (ferredoxin NADP reductase); (2) Cyt b_6/f; or (3) other electron acceptors, from a network of alternative electron pathways, such as O_2, which is reduced to O_2^-, in the water-water cycle (WWC); subsequently O_2^- is detoxified to H_2O. ATP is produced by the enzyme CF_1–CF_0 ATP synthase from adenosine diphosphate (ADP) and inorganic phosphate (P_i), using the proton motive force (pmf), made up of the electrical potential ($\Delta\Psi$) and the proton gradient (ΔpH) built across the thylakoid membrane (with the proton flux originating from the water splitting at the OEC, and the cyclic reduction–oxidation of PQ/PQH_2). Finally, ATP and NADPH are used in Calvin–Benson cycle to fix CO_2 from the atmosphere in carbohydrates. Modified from Govindjee et al. (2010); it also includes information from Stirbet and Govindjee (2011), Cramer and Zhang (2006), and Baniulis et al. (2008)

The interpretation of experimental results obtained with different types of fluorometers is currently another challenge. Therefore, in order to understand the relationship of Chl *a* fluorescence to various photosynthetic processes, parallel measurements of several reactions are expected to provide better insight into their mechanisms. In this sense, the development of instruments capable of measuring simultaneously the prompt fluorescence and several other types of signals, such as 820 nm transmission changes (related to the PSI activity), and the delayed fluorescence (in the µs–ms range, related to the back reactions of PSII), offers new opportunities for the improvement of the analysis of the relationship of fluorescence with other photosynthetic processes (Schreiber and Schliwa 1987; Schreiber et al. 1988; Schreiber and Neubauer 1989, 1990; Klughammer and Schreiber 1998, 2008; Schreiber 2002, 2004; Schansker et al. 2003, 2005, 2008, 2011; Goltsev et al. 2009). However, for a quantitative understanding of these

Fig. 2 Chlorophyll *a* fluorescence induction transients from a pea leaf (kept in darkness for 20 min before the measurement). They are plotted on a logarithmic time scale (**a**); and on a linear time scale (**b**). Wavelength of excitation: 650 nm. Excitation light intensity, at the leaf surface, for curves labeled *1*, *2* and *3* was 32, 320, and 3,200 μmol photons m^{-2} s^{-1}. For definition of OJIPSMT symbols, see the text. Fluorescence is normalized at F_0. *Source*: Strasser et al. (1995); modified by Alaka Srivastava

relationships, we would also need parallel measurements on the key intermediates (e.g., pheophytin and Q_A), as well as on the end products of the reactions (O_2 evolution and CO_2 fixation). In general, various fluorescence kinetic techniques, developed recently, have begun to provide both qualitative as well as quantitative information on a large variety of photosynthetic events. There are numerous applications in very different areas, from fluorescence analysis under field conditions (Logan et al. 2007; Cessna et al. 2010) to aquatic sciences (Kolber et al. 1998; Schreiber 1998; Suggett et al. 2010), from screening mutants (Codrea et al. 2010) to cellular and molecular analysis (Xu et al. 1990; Oxborough and Baker 1997; Schreiber 1998; Goth et al. 1999; Osmond et al. 1999; Snel and Dassen 2000; Küpper et al. 2000), ultimately reaching planetary dimensions through remote sensing methods (Flexas et al. 2002; Moya and Cernovic 2004; Kolber et al. 2005).

At the very outset, we refer the readers to the many reviews on Chl *a* fluorescence, published during the last 45 years: Butler (1966), Govindjee and Papageorgiou (1971), Mohanty and Govindjee (1973), Papageorgiou (1975), Govindjee and Jursinic (1979), Renger and Schreiber (1986), Schreiber (1986, 2004), Briantais et al. (1986), Horton and Bowyer (1990), Krause and Weis (1991), Dau (1994), Govindjee (1995, 2004), Lazár (1999), Maxwell and Johnson (2000), Rosenqvist and van Kooten (2003), Strasser et al. (2000, 2004), Joshi and Mohanty (2004), Lazár (2006), Osmond and Forster (2006), Rottgers (2007), Papageorgiou et al. (2007), Baker (2008), Roháček et al. (2008), Lazár and Schansker (2009), Papageorgiou and Govindjee (2011), Papageorgiou (2011); Forster et al. (2011) and Stirbet and Govindjee (2011); and to several books: Govindjee et al. (1986), Ke (2001), Blankenship (2002), Papageorgiou and Govindjee (2004), Falkowski and Raven (2007), Laisk et al. (2009a, b), and Eaton-Rye et al. (2011).

Fast fluorescence induction: early interpretations by L. N. M. Duysens, R. Delosme, and P. Joliot

Duysens and Sweers (1963) explained the FI based on their detailed two-light experiments (that had followed the previous preliminary observations by Govindjee et al. 1960; see comments in Govindjee and Seufferheld 2002). They suggested the concept that PSII reduces a quencher of fluorescence "Q", later identified as the bound plastoquinone Q_A in its oxidized state (van Gorkom et al. 1978; van Gorkom 1986), while PSI oxidizes the reduced quencher, labeled "QH". Duysens and Sweers (1963) proposed the following reaction scheme (rewritten in today's language):

$$
\begin{aligned}
&H_2O \rightarrow (\text{photosystem II}) \rightarrow Q_A \rightarrow \text{Cyt } b_6/f \\
&\quad \rightarrow (\text{photosystem I}) \rightarrow A_0 \rightarrow NADP^+ \rightarrow CO_2
\end{aligned}
$$

where Cyt b_6/f is the cytochrome b_6/f complex, A_0 is the primary electron acceptor of PSI (a special Chl *a* molecule), $NADP^+$ is nicotinamide adenine dinucleotide phosphate, and CO_2 represents the final electron acceptor in the Calvin–Benson cycle (see Fig. 1). The main idea of Duysens and Sweers (1963) was that the fluorescence yield increases as PSII RCs become 'inactive' ("closed"), i.e., as the photochemical reduction of Q_A takes place.

We note that, in 1963, the pheophytin (Phe) was not yet discovered, and Q_A was considered the primary electron acceptor of PSII. Moreover, Duysens and Sweers (1963) suggested that the relationship between the fluorescence yield, *F*, and number of photoreduced Q_A is linear since Q (now called Q_A) was considered to be the only factor influencing PSII fluorescence yield. Further, they explained the OI(D)P transient as follows: the fluorescence rise is mainly due to the net reduction of Q_A, but the inflection may be due to PSI producing an oxidized intermediate that oxidizes the reduced Q_A; this PSI effect soon stops because of the exhaustion of this intermediate, and the fluorescence rises to its maximum P level; thus, it is the interaction of PSI and PSII

that is responsible for the details of the OI(D)P transient (for an earlier suggestion, see Kautsky et al. 1960). Munday and Govindjee (1969a) were the first to substantiate experimentally the idea that PSI activity influences the I–P phase of the fast FI, and showed it to be the cause of the dip D. They suggested that, in saturating light, the P level is reached due to the complete reduction of Q_A and intersystem intermediates, caused by the development of a block in the oxidation of the reduced electron acceptors in PSI. Further, they specifically stated that at "P", there was a "traffic jam" of electrons at the electron acceptor end of PSI.

Morin (1964) was the first to measure the fast FI with a high resolution at high light intensities using an original homemade fluorometer. In order to illuminate the sample quickly, he used a "gun" to move the shutter out of the beam. He showed that, in saturating light, fluorescence rises rapidly from the minimum O to an intermediary level that he called I (equivalent to the J level in the current nomenclature; Strasser and Govindjee 1991, 1992). Further, he observed that the amplitude of this phase depends strongly on the number of photons absorbed by the sample. This clearly suggested that the O to I (= J) rise is the "photochemical phase". The subsequent slower I (= J)–P rise was less dependent on light intensity but it was quite sensitive to temperature, disappearing at subfreezing temperatures; thus, Morin labeled it as "thermal phase". In this review we will use the terms "photochemical phase" and "thermal phase" independent of the theories used for their description, for the O–J rise and J–I–P rise measured *under saturating light conditions*.

Based on experimental results obtained with the fluorometer of Morin, Delosme (1967) suggested a different interpretation of the fast fluorescence transient than that given by Duysens and Sweers (1963). Since measurements were made with saturating light, Delosme (1967) stated that during the photochemical phase (i.e., the O–I (= J) rise), the quencher "Q" (i.e., Q_A) of Duysens and Sweers is completely reduced, but the fluorescence is still low due to the presence of a second hypothetical quencher, R, on the acceptor side of PSII (i.e., the secondary plastoquinone Q_B, or the oxidized PQ-pool—see below for different theories). Further, during the thermal phase (i.e., the I (= J)–P rise), the fluorescence would increase until reaching a maximum value due to the removal (consumption) of the quencher R. However, the quencher R may, instead, be taken to represent an unknown process that is somehow connected with a part of PSII that can influence the fluorescence yield of antenna Chls. Clearly, these concepts have remained vague. Further, Delosme (1967) suggested that the thermal phase is absent in fluorescence transient measured on samples treated with DCMU (3-(3′,4′ dichlorophenyl)-1,1′ dimethylurea), when the electron transfer from reduced Q_A to Q_B is blocked (see e.g., Vermaas et al. 1984).

Joliot and Joliot (1973, 1977, 1979, 1981a, b) (see also comments in Vermaas and Govindjee 1981) advanced a different idea than Delosme, but included the concept of two quenchers responsible for the fluorescence transient. For example, in their view, contrary to the opinion of Delosme (1967), the second quencher would influence also the fluorescence transient of samples treated with DCMU. Joliot and Joliot (1977) measured, in parallel, Chl *a* FI in the presence of DCMU and an absorption change at 550 nm (ΔA_{550}) after several short-saturating flashes. It is known that the ΔA_{550} (C550) is due to a blue shift in pheophytin (Phe) absorbance, suggested to be induced by the electrical charge on reduced Q_A; thus, C550 was used as an indirect measure of the degree of Q_A reduction (Erixon and Butler 1971; Klevanik et al. 1977; Melis and Schreiber 1979). Joliot and Joliot observed that this ΔA_{550} reached almost its highest value already after one saturating flash, but the fluorescence yield was only ~70–75 % of the maximum variable fluorescence, $F_V = F_M - F_0$. Several more flashes were needed before the maximum fluorescence yield was reached. Yet, spectroscopic measurements indicated that ~90 and ~97 % of Q_A was already reduced after the first and second flashes (Joliot and Joliot 1981b). The Joliots explained these results by assuming the presence of another quencher, which they called Q_2 (Q_1 being reserved for Q_A).

Joliot and Joliot (1977, 1979) proposed a model that explained the above results by assuming the existence of a *double hit* process during a high intensity light flash: after the *first hit* Q_A is reduced, and the oxidized primary PSII donor, $P680^+$, receives an electron from the secondary electron donor Y_Z, in a short time compared to the duration of the flash; this would allow a *second hit* that would reoxidize P680 and reduce the hypothetical quencher Q_2. Finally, the Joliots suggested that $P680^+$ would be reduced by another (auxiliary) donor, labeled D, working less efficiently than Y_Z (see Fig. 3; modified after Joliot and Joliot 1977, 1979).

The observation of a component in the delayed fluorescence, presumably caused by a back reaction between Q_A^- and $P680^+$, which is insensitive to the membrane potential (Jursinic et al. 1978), was explained to arise by a back reaction between Q_2^- and $P680^+$. Joliot and Joliot (1981a) concluded that, after the photoreduction of Q_2, the charge stabilization is less efficient, due to a slower reduction of $P680^+$ by the donor D, and a faster back-reaction between Q_2^- and $P680^+$. However, they also proposed another possible model explaining their experimental results, where all the PSII centers included both Q_A and the hypothetical Q_2, but only a fraction of them had Q_2 connected to Q_A. The path of electron flow from P680 to Q_2 and to Q_A was considered to take place as follows: (1) when Q_2 is connected to Q_A, the latter accepts electrons

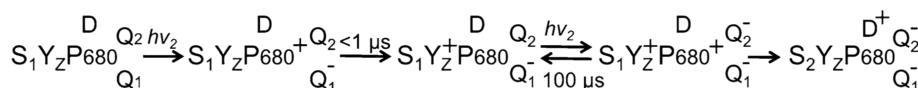


Fig. 3 The sequence of reactions at the reaction center (RC) of photosystem II (PSII) after illumination with a saturating single turnover flash (STF) of 2 µs duration. This diagram shows the 'double hit' *model* of Joliot and Joliot (1977, 1979). S_1 and S_2 are the two redox states of the oxygen evolving complex (OEC); Y_Z is the secondary electron donor to the oxidized P680 ($P680^+$) of PSII; D is another electron donor to the oxidized P680 ($P680^+$) of PSII (Joliot and Joliot 1977); P_{680} is the primary electron donor of PSII; Q_1, usually labeled as Q_A, is the primary plastoquinone acceptor of PSII; and Q_2 is a hypothetical electron acceptor that receives electrons from P_{680} (via Phe), in parallel with Q_1. Modified from Joliot and Joliot (1979)

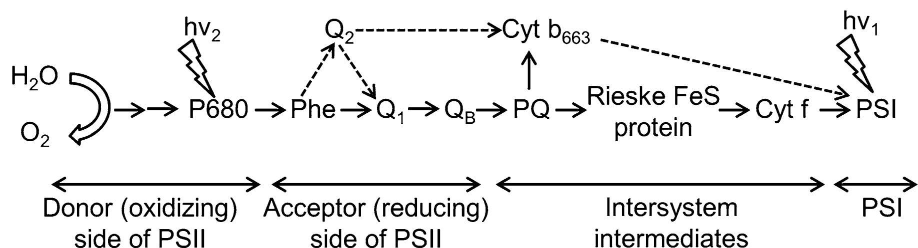


Fig. 4 An alternate sequence of reactions taking place upon illumination with a saturating single turnover flash (STF) (also of 2 µs duration; cf. with Fig. 3). This diagram is based on the 'two-acceptor' model of Joliot and Joliot (1981b). P_{680} is the primary electron donor of Photosystem II (PSII); Phe is pheophytin, the primary electron acceptor of PSII; Q_1, usually labeled as Q_A, is the primary plastoquinone acceptor of PSII; Q_2 is a hypothetical electron acceptor of PSII that receives electrons from Phe, in parallel with Q_1; Q_B, is the secondary plastoquinone acceptor of PSII; PQ represents a pool of mobile plastoquinone molecules; Cyt b_{563}, Rieske Fe–S protein, and Cyt f are components of the Cytochrome b_6/f Complex; PSI is photosystem I. Modified from Vermaas and Govindjee (1981)

efficiently from the reduced Q_2; (2) in centers where Q_2 is not connected to Q_A, Q_2^- is assumed to be stable only in the presence of reduced Q_A, and to donate electrons only to cytochrome Cyt b_{563} in the Cyt b_6/f complex (see Fig. 4). At one time, Q_2 was suggested (Joliot and Joliot 1981a, b) to be identical to an undetermined intermediate labeled "X_a" (probably Phe_{D2}), as its reduction does not lead to an absorption change at 515 nm (ΔA_{515}) that lasts longer than a few µs (Eckert and Renger 1980).

Other emerging views

The above-mentioned model proposed by Joliot and Joliot (1979, 1981a, b) remains speculative, as the nature of Q_2 is not yet known, and further, with the present knowledge of PSII structure (see e.g., Umena et al. 2011), there seems to be no room for an additional electron acceptor. However, Boussac et al. (2011), based on measurements by EPR spectroscopy at low temperature, coupled with absorption spectroscopy at 552 nm, have identified the non-heme iron (NHI) as a possible candidate for Q_2. They have shown that samples kept for some time in darkness contain a fraction of PSII centers with NHI in oxidized state (i.e., with Fe^{3+}), which in the presence of DCMU is higher in amount than in its absence. Boussac et al. (2011) determined that during the first flash of light, all Q_A molecules are rapidly reduced, but subsequently part of them transfer an electron to the oxidized NHI with a high rate ($\tau_{1/2} \approx 50$ µs in PsbA3-PSII of *Thermosynechococcus elongatus*, and $\approx$25 µs in higher plants; Petrouleas and Diner 1987). Therefore, the maximum fluorescence amplitude after one flash (F_M^{STF}) would be lower than the maximum fluorescence caused by a saturating continuous excitation (F_M), not because of the existence of a second quencher, but due to the rapid reoxidation of a fraction of Q_A^- by the oxidized NHI. We note that the reoxidation of Q_A^- by NHI would have practically no influence on O–J–I–P curves because, in continuous light, Q_A will be re-reduced until the fluorescence reaches its maximum (F_M). This way, the identification of oxidized NHI as Q_2 could be easily considered consistent with the theory of Duysens and Sweers, in its modified version, as presented later in this review. Still, the reoxidation of NHI was shown to take much longer than the time needed to reach F_M—actually seconds—under normal conditions (Diner and Petrouleas 1987). Hence, after the second flash, the fluorescence yield would have to reach already its maximum value (F_M), while the experiments with DCMU-treated samples show that several flashes are necessary to reach the maximum (Joliot and Joliot 1977, 1979, 1981b; Vredenberg et al. 2006). A possible explanation of these results could be, according to Lavergne and Rappaport (1998), a direct $P680^+$ quenching (since after a second hit its reduction by Y_Z is slowed down), and partial reopening of RCs by rapid charge recombination of $P680^+$ with Q_A^-. We note that direct quenching of Chl fluorescence by $P680^+$ can potentially play a more important role in the experiments using light flashes, if we accept the suggestion of Steffen et al. (2005) that the quenching by $P680^+$ is higher by a

factor of two in comparison to the quenching by Q_A. [For early papers on quenching of Chl fluorescence by $P680^+$, see references in the next section.]

Schreiber and Neubauer (1987) (see also Neubauer and Schreiber 1987), using a new type of fluorometer, equipped with an array of emitting diodes (Schreiber 1986), were able to measure the fast FI in high light with a good time resolution. They confirmed the results obtained by Morin (1964) and Delosme (1967) relative to the properties of the photochemical and thermal phases, and advocated the hypothesis that assumes almost complete reduction of Q_A at the end of the O–J phase of fluorescence transient measured in saturating light, and a different origin for the J–I–P fluorescence rise than Q_A reduction. During the last 25 years, several research groups have attempted to find experimental arguments supporting this interpretation of the fast FI. They have tried to use various hypothetical mechanisms for the thermal phase, related either to the quencher R, proposed by Delosme, or to the quencher Q_2, proposed by Joliot and Joliot, or other non-photochemical processes enhancing the fluorescence yield (see a review by Samson et al. 1999 and references therein).

In this review we will analyze different models regarding the origin of the thermal phase, presenting new available data since the review of Samson et al. (1999). Further, we will include here additional arguments based on results obtained from mathematical simulations. As a background for our review, we will first briefly describe factors other than Q_A that are known to affect the fluorescence yield during the O–J–I–P transient.

Modulation of the fast FI by processes or components of the photosynthetic apparatus other than Q_A

It is now well known that, besides Q_A, a number of other endogenous components (e.g., $P680^+$; Y_Z^+; Phe^-; PQ; and even Q_B; Fig. 1) or processes, act, or have been suggested to act, as quenchers or modifiers of the fast (up to a second) fluorescence transient (see Dau 1994; Kramer and Crofts 1996).

A list of the known quenchers of Chl a fluorescence: a necessary background for understanding the O–J–I–P fluorescence transient

The quenchers are:

(1) $P680^+$ can quench Chl *a* fluorescence as efficiently as Q_A (Okayama and Butler 1972; Butler et al. 1973; Shinkarev and Govindjee 1993; Bruce et al. 1997; for a different opinion regarding $P680^+$ efficiency as a quencher, see Steffen et al. 2005).

(2) P680 triplet, $^3P680$ (most likely $^3Chl_{D1}$ in equilibrium with $^3P_{D1}$; Renger and Schlodder 2010) has a lifetime of hundreds of µs and is a strong quencher of Chl *a* fluorescence (Barzda et al. 2000).

(3) Carotene triplet ^3Car (lifetime of the order of 5–10 µs) is also a quencher of Chl *a* fluorescence formed upon excitation of the sample with high energy flashes (Duysens et al. 1972, 1975; Zankel 1973; Mauzerall 1976; Barzda et al. 2000).

(4) Long-lived quenchers (lifetime longer than a few ms), originating probably from Chl cations, or other radicals that are produced either directly from Chl triplets, or via the singlet oxygen generated in reactions sensitized by the Chl triplet (Barzda et al. 2000) must also be included.

(5) Non-photochemical quenching by oxidized PQ molecules in the PQ-pool (Vernotte et al. 1979; Kramer et al. 1995; Prasil et al. 1996; Samson and Bruce 1996; Vasilev and Bruce 1998; Koblizek et al. 2001; Haldimann and Tsimilli-Michael 2005). The degree of quenching is lower for open than for closed PSII centers (i.e., 7 %, compared with 30 %; Vernotte et al. 1979). It is easily observed in samples treated with inhibitors that block the reduction of the PQ-pool (e.g., DCMU), when compared with untreated samples. Tóth et al. (2005) showed that the oxidized PQ-pool did not quench fluorescence in leaves when the DCMU treatment was administered slowly overnight; this may suggest that non-photochemical quenching by oxidized PQ-pool is present only when the structural integrity of the photosynthetic apparatus is compromised.

(6) $Chlz_{D1}^+$ was shown to be a very strong fluorescence quencher (Buser et al. 1992; Schweitzer and Brudvig 1997). $Chlz_{D1}^+$ participates in cyclic electron transport around PSII (CET-PSII), being involved in the photoprotection process under high light (Shinopoulos and Brudvig 2011). There is evidence for the accumulation of Chl^+ at physiological temperatures in samples where low pH inhibits the donor side of PSII. The lifetime and yield of Chl cations ($P680^+$ and/or Chl_Z^+), formed after a single turnover flash (STF) in PSII enriched thylakoid membranes, was shown to increase at low pH, and this was correlated with increased fluorescence quenching (Bruce et al. 1997).

(7) Phe_{D1}^- was reported to be a fluorescence quencher by Klimov et al. (1977, 1978); its accumulation takes place only under irradiation at 200–220 K (Klimov et al. 1980; Breton 1982), or under anaerobic conditions (Klimov et al. 1985, 1986). (For a different opinion, see Vredenberg 2000, 2004.)

A list of a number of processes that affect the fast fluorescence induction

Processes affecting the fast FI are:

(1) NHI—in its oxidized state—can accept electrons from reduced Q_A, and therefore influence the fluorescence induced after a flash (Diner and Petrouleas 1987; Petrouleas and Diner 1987; McConnell et al. 2011; Boussac et al. 2011).

(2) The redox states of OEC (i.e., the so-called S-states) are responsible for flash-number dependent oscillations in experiments with a sequence of STFs (Delosme 1971; Duysens et al. 1975; Shinkarev and Govindjee 1993; Rappaport et al. 1994; Delosme and Joliot 2002; Jablonsky and Lazár 2008, Jablonsky et al. 2008), but they can also influence the fast FI transient in continuous light (Hsu 1993; Lazár 2003; Gauthier et al. 2010).

(3) The degree of energetic connectivity between PSII units affects the rate constant of Q_A reduction, and therefore the fluorescence transient. Joliot and Joliot (1964) were the first to show a hyperbolic relation between the relative variable fluorescence, $V(t) = (F_t - F_0)/(F_M - F_0)$, and the fraction of closed PSII centers, $B(t) = [Q_A^-](t)$ (see Eq. 1), with the hyperbolic constant dependent on the so-called 'connectivity constant' p. Strasser (1978, 1981) has shown that this constant can be expressed as a product of two distinct terms, the first depending on the energy influx at the two extremes (i.e., with all PSII RCs either open or closed), and the second, upon the probability of energetic communication among the PSII pigment pools (see also Strasser et al. 2004).

(4) PSI activity was shown to influence the thermal phase of the fast FI (Munday and Govindjee 1969a, b; Schreiber and Vidaver 1974; Satoh 1981; Hansen et al. 1991; Schansker et al. 2003, 2005).

(5) Ferredoxin-NADP$^+$-reductase (FNR), which is usually inactivated in plants kept in darkness for some time, may become activated during the fast FI, influencing PSI electron transport kinetics and the thermal phase in some plants (e.g., lichen thalli containing *Trebouxia erici*; Ilik et al. 2006); however, in studies on isolated *Asterochloris erici*, such a correlation between PSI electron transport kinetics and the shape of the thermal phase was not found (personal communication from A. Guéra (2012): unpublished work of F. Gasulla, G. Salvà, Z. Nocete, E. Barreno and A. Guéra). (Light activation of FNR influences usually the fluorescence yield during the slow FI; Schansker et al. 2006.)

(6) PSII heterogeneity influences the fluorescence transient since various types of PSII (e.g., PSIIα, PSIIβ, Q_B-nonreducing PSII, inactive PSII; Melis and Homann 1975, 1976; Cao and Govindjee 1990; Govindjee 1990; Lavergne and Leci 1993) affect fluorescence yield in different ways.

(7) Cyclic electron transport around PSI, CET-PSI (see Joliot et al. 2006; Shikanai 2007; Laisk et al. 2007, 2010; Joliot and Johnson 2011; Johnson 2011), may also affect the fast FI. Lazár (2009), based on mathematical simulation of the O–J–I–P transient, has considered a direct influence of CET-PSI on the fluorescence transient, which is much more significant at lower, than higher, light intensities. On the other hand, Vredenberg (2011) has postulated a significant non-photochemical fluorescence enhancement during the I–P phase, induced by the membrane potential ($\Delta\Psi$) generated by CET-PSI.

(8) Mehler reaction, or water–water cycle (WWC), and alternative ET pathways, which regulate electron transport (Asada 2000, 2006; Peltier et al. 2010; Okegawa et al. 2010; Hemschemeier and Happe 2011; McDonald et al. 2011; Cardol et al. 2011), also affect Chl *a* fluorescence. Such reactions function as electron sinks, increasing the acceptor pool size, and therefore also the area over the fast FI curve (which is usually used as a measure of the number of turnovers that are required for RC closure; Malkin 1966; Malkin and Kok 1966; Murata et al. 1966).

(9) Cyclic electron transport around PSII, CET-PSII, is also expected to influence Chl *a* fluorescence (Lazár et al. 2005). Indeed, in intact chloroplasts CET-PSII was demonstrated to be almost equal to the WWC electron flow, implying that it can effectively dissipate excess light energy and contribute to photoprotection of PSII under conditions that limit photosynthesis (Thompson and Brudvig 1988; Miyake and Yokota 2001; Miyake et al. 2002; Shinopoulos and Brudvig 2011).

(10) Local pH, or transmembrane proton gradient, ΔpH, affects the rate constants of some electron transport reactions that are pH sensitive (Wraight and Crofts 1970). (Additionally, the P to S decline of fluorescence transient has been related to pH changes; see Briantais et al. 1979.)

(11) Divalent cations enhance Chl *a* fluorescence yield, whereas monovalent cations cause the opposite effect (Homann 1969; Murata 1969a, b; Gross and Hess 1973; Butler and Kitajima 1975; Butler and Strasser 1977b; Wong and Govindjee 1979, 1981; Barber 1980; Mehta et al. 2011; Papageorgiou and Govindjee 2011).

(12) Local electric fields (e.g., the electric field generated around a component of the photosynthetic electron transport chain, or a group of components situated in close vicinity also affect fluorescence). The membrane potential ($\Delta\Psi$) is expected to affect Chl *a* fluorescence for various reasons, including the Stark effect (Diner and Joliot 1976; Graan and Ort 1983; Bulychev et al. 1986; Bulychev and Niyazova 1989; Zheng et al. 1990; Dau et al. 1991; Dau and Sauer 1991, 1992; Bulychev and Vredenberg 1999; Pospíšil and Dau 2002; Vredenberg and Bulychev 2002, 2003; Belyaeva et al. 2003; Rubin and Riznichenko 2009; Antal et al. 2011) (see "Understanding the thermal phase: an integrated view" section).
(13) Conformational changes influencing the fluorescence yield have been suggested to take place during the fast FI (Bradbury and Baker 1983; Moise and Moya 2004a, b; Schansker et al. 2011).

In a large number of studies on the fast FI, these additional fluorescence quenchers and modulators have been neglected (see e.g., the so-called JIP-test, pioneered by Strasser and Strasser 1995; Strasser et al. 2000, 2004; Stirbet and Govindjee 2011), in order to allow a simplified theoretical approach. However, their consideration can have significant effects on conclusions obtained under some experimental conditions.

Duysens and Sweers (1963) theory revisited

It is important to note that Duysens and Sweers (1963) had assumed in their original theory that, in dark adapted samples, the fluorescence rises from F_0 to F_P only under the influence of one factor: disappearance of the fluorescence quencher Q (i.e., Q_A). Consequently, in their hypothesis, a linear relationship between the fluorescence F and the number of closed active PSII centers (i.e., with reduced Q_A) was assumed. However, in reality, this linear relationship does not exist, due to the influence of fluorescence quenchers and modifiers mentioned above. A major parameter is PSII connectivity, which leads to a hyperbolic relationship between the relative variable fluorescence $V = (F_t - F_0)/(F_M - F_0)$ and the fraction of closed PSII centers $B = [Q_A^-]$ (Joliot and Joliot 1964; see Eq. 1). Therefore, the original theory of Duysens and Sweers (1963) must be modified in order to account for the presence of more than one factor affecting the fluorescence yield. We call the modified theory that we have adopted in this paper as: 'A modified version of Duysens and Sweers Theory'.

A modified version of Duysens and Sweers Theory

The features of this version of Duysens and Sweers theory are:

(1) Q_A is the main quencher influencing the fluorescence yield during the O–J–I–P transient in dark adapted samples; Q_A is gradually reduced, in parallel with the fluorescence rise from F_0 to F_P. In saturating light, Q_A is completely reduced, in all the active PSII centers, at the moment when fluorescence reaches the F_M level (as was assumed in the original theory of Duysens and Sweers 1963).
(2) Other fluorescence quenchers and modifiers (see above) can affect the fluorescence transient; thus, the relationship between F and $[Q_A^-]$ is not linear.
(3) At the F_M level, besides the removal of the photochemical quenching (i.e., the quenching of fluorescence by Q_A), all other types of non-photochemical quenching (or enhancement) that affect the fluorescence yield during the O–J–I–P(= M) rise must also be removed. Consequently, F_M has the same value to that resulting from the complete reduction of Q_A, in all the active PSII centers, as in the hypothesis that Q_A is the only quencher (i.e., as in the original theory of Duysens and Sweers 1963). [See details about the reasons behind this hypothesis later, in the section where we discuss the maximum quantum yield of primary PSII photochemistry, Φ_{PSII}.]

In view of the above discussion, we conclude that the theory of Duysens and Sweers, in its modified version, supports the idea that in order to reach the maximum fluorescence yield (F_M), *it is necessary, and sufficient*, to have Q_A completely reduced in all the active PSII centers.

However, the presence of multiple fluorescence quenchers and modifiers has also inspired models based on another kind of 'adjustment' to the original theory of Duysens and Sweers (1963), which are 'alternatives' to the modified version of Duysens and Sweers Theory. The main concepts advocated in these alternative theories are presented below.

Alernatives to the modified version of Duysens and Sweers theory

The main concepts of the alternative theories are:

(1) In order to reach the maximum fluorescence yield (F_M), *it is necessary, but not sufficient* to have Q_A completely reduced in all the active PSII centers. Many variations of this type of theory have been proposed, starting with Delosme (1967) who suggested that, in saturating light, Q_A is completely

reduced in all the active PSII centers before the fluorescence reaches its maximum, but the fluorescence at that moment is lower than F_M, due to the presence of a second quencher (R), as mentioned earlier, so that F_M is attained only when this quencher is also consumed.

(2) Several other fluorescence quenchers and modifiers can affect the fluorescence transient; thus, the relationship between F and $[Q_A^-]$ is not linear.

(3) While some of the alternative theories (e.g., Delosme 1967; Joliot and Joliot 1979, 1981a, b; Schreiber et al. 1989, Samson and Bruce 1996; Schreiber 2002) are in agreement with the above-described feature (3) of the modified version of Duysens and Sweers Theory, others are not (e.g., Schreiber and Krieger 1996; Bulychev and Vredenberg 2001; Pospíšil and Dau 2002; Vredenberg 2008a, 2011; Schansker et al. 2011). Indeed, in these last theories it is assumed that a non-photochemical fluorescence enhancement contributes to the maximum fluorescence F_M, in addition to the contribution due to the complete Q_A reduction in active PSII centers.

A major goal of what follows in this review is to discover which of these variations of the original theory of Duysens and Sweers (1963) is most credible. And we stress here that the conclusions of our analysis represent our personal point of view on this subject. Since the assumed origin of fluorescence rise during the thermal phase (i.e., the J–I–P phase) differs in each of these theories, our review is focused on our "personal perspective of the thermal phase, the J–I–P rise" (the subtitle of our review).

Interpretation of the fluorescence induction O–J–I–P based on the modified version of Duysens and Sweers Theory: mathematical modeling

An important theoretical approach to confirm the interpretations of the fast FI is the mathematical modeling of fluorescence transients. This can be done by using models based on generally accepted structural and functional information (e.g., biological, biochemical and biophysical) obtained from specific samples (e.g., leaf, individual cells, intact chloroplasts, thylakoids). This criterion, of what might be called 'structure-based modeling', is not fulfilled in some studies, in which the experimental curves were fitted with analytical functions that were not deduced from structural information (see e.g., Pospíšil and Dau 2000, 2002; Boisvert et al. 2006; Antal and Rubin 2008; Joly and Carpentier 2009; and also, partially, in the models of Vredenberg 2008a, Vredenberg and Prášil 2009, Vredenberg 2011).

The idea of a quantitative mathematical description of the fluorescence transient from F_0 to F_P, was first used by Malkin and Kok (1966) and Malkin (1966), and by Munday and Govindjee (1969a), both groups accepting the hypothesis of Duysens and Sweers (1963) that the redox state of Q_A controls the fluorescence yield. However, Munday and Govindjee (1969a), based on an idea formulated by Kautsky et al. (1960), were the first to show experimentally, as mentioned earlier, that the OIDP transient is influenced also by events taking place at the PSI level; their results and conclusions have been supported by several studies (Schreiber and Vidaver 1974; Satoh 1981; Hansen et al. 1991; Schansker et al. 2003, 2005).

Advancement in the understanding of the structure and function of PSII (see Fig. 1) has provided information that formed the theoretical basis for the modeling of Chl *a* FI. These include: S-state cycle of the oxygen evolving complex (OEC) (Kok et al. 1970); reversible radical pair (RRP) model describing the ultrafast steps of exciton trapping and charge stabilization in PSII RC (Schatz et al. 1988; Trissl et al. 1993; Trissl and Lavergne 1995; Lavergne and Trissl 1995; Trissl 2002; Dau 1994) the two-electron gate (TEG) mechanism related to the reduction of Q_A and Q_B on the electron acceptor side of PSII (Velthuys and Amesz 1974; Crofts and Wraight 1983); and the reoxidation of plastoquinol PQH_2 at the Cyt b_6/f level (Crofts 2004; Cramer and Zhang 2006; Baniulis et al. 2008).

The rapid development of computers in the 1980s increased the opportunity to simulate the fast FI curves, starting with the pioneering work of Renger and Schulze (1985), which used an extended TEG model (see Fig. 5) to simulate and fit several OIDP fluorescence transients measured at different low light intensities. At the same time, Sorokin (1985) simulated the FI in DCMU-treated chloroplasts. We note that Renger and Schulze (1985) had also considered the heterogeneity of PSII antenna and different PQ-pool sizes. Their conclusion was that the concept of Q_A fluorescence quenching is fully supported under the illumination conditions they had used. For a comprehensive review on mathematical modeling of the fast fluorescence kinetics, see Lazár and Schansker (2009). In the following section we will comment on some of the representative structure-based mathematical models developed for the simulation of the O–J–I–P transient, which provide results in favor of the modified version of Duysens and Sweers Theory, and are related to the specific topic of our review: *the interpretation of the J–I–P rise (i.e., thermal phase) of the fast FI.*

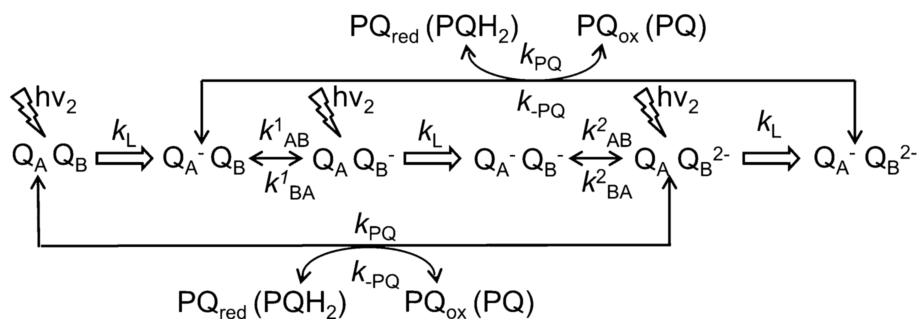


Fig. 5 A simplified reaction scheme of the two electron gate (TEG) model, describing the photoreduction of the acceptor side of PSII. Q_A is the primary plastoquinone electron acceptor; Q_B is the secondary plastoquinone electron acceptor; PQ is a plastoquinone molecule from the PQ-pool; PQH_2 is a plastoquinol molecule from the PQ-pool; k_L is the apparent rate constant of Q_A photoreduction; $k^1{}_{AB}$ and $k^1{}_{AB}$ are the forward and backward rate constants of the reduction of Q_B; $k^2{}_{AB}$ and $k^2{}_{BA}$ are the forward and backward rate constants of the reduction of $Q_B{}^-$; k_{PQ} and k_{-PQ} are the forward and backward rate constants of PQH_2/PQ exchange. Modified from Renger and Schulze (1985)

Mathematical simulation of the O–J–I–P transient: Is it possible to describe both the photochemical and thermal phases based on the modified version of Duysens and Sweers Theory?

One of the simplified reaction schemes used for the simulation of O–J–I–P curves is the TEG model, in which Q_A, Q_B, and PQ-pool are the only components of the electron transport chain that are explicitly considered (see Fig. 5) (Baake and Strasser 1990; Hsu 1992; Stirbet and Strasser 1995, 1996; Strasser and Stirbet 1998; Goltsev and Yordanov 1997; Lazár et al. 1997; Tomek et al. 2001, 2003; Sušila et al. 2004). The Ordinary Differential Equation (ODE) system describing the kinetics of these reactions (see the reaction scheme in Fig. 5) is solved by numerical methods, using initial values of the components and rate constants from the literature. The relative variable Chl *a* fluorescence $V(t)$ for the case of connected PSIIs can be calculated using the following equation (Joliot and Joliot 1964; Strasser 1978, 1981; Butler 1980; Trissl and Lavergne 1995; Lavergne and Trissl 1995):

$$V(t) = \frac{F(t) - F_0}{F_M - F_0} = \frac{B(t)}{1 + C_{HYP} \cdot [1 - B(t)]} \quad (1)$$

where $B(t)$ is the simulated value of the fraction of PSII units with Q_A reduced, $B(t) = [Q_A{}^-](t)$, and C_{HYP} is the hyperbolic constant, that depends on the so-called 'connectivity parameter' p, the probability of energetic communication among the PSII pigment pools (see e.g., Strasser 1978, 1981 for the relationship between C_{HYP} and p). This relation has been used in fast FI simulations by, e.g., Stirbet et al. (1998) and Lazár et al. (2001). Further, the apparent rate constant of Q_A reduction, k_L, is in this case variable in time (see details in Strasser et al. 2004) and can be calculated as:

$$k_L(t) = k_L^0 \cdot \frac{1 + C_{HYP}}{1 + C_{HYP} \cdot [1 - B(t)]} \quad (2)$$

where k_L^0 is the initial rate constant of Q_A reduction (i.e., at the O level). However, for extended TEG models, in which the ultrafast part of exciton trapping is also explicitly considered by means of the RRP model, equations equivalent to Eqs. 1 and 2 can be obtained based on specific parameters of the model (see e.g., Baake and Schlöder 1992). In the particular case of $C_{HYP} = 0$, i.e., when the connectivity is ignored in the model, the relative variable fluorescence becomes identical to the fraction of closed RCs, $V(t) = B(t) = [Q_A{}^-](t)$, and the apparent rate constant of Q_A reduction is then constant in time, $k_L(t) = k_L^0$. We emphasize that both Eqs. 1 and 2 must be used to obtain a complete picture.

Different extended TEG models have been proposed (e.g., by Renger and Schulze 1985; Baake and Schlöder 1992; Hsu 1993; Stirbet et al. 1998; Strasser and Stirbet 2001; Lebedeva et al. 2002; Lazár 2003; Zhu et al. 2005), in which other components of PSII are also explicitly considered in the reaction scheme, or different processes affecting the electron transfer reactions or the fluorescence yield are taken into account, as for example: excitation energy transfer and primary charge separation and stabilization (as described by the RRP model of Schatz et al. 1988; Leibl et al. 1989; Trissl et al. 1993; see a discussion of this model in Dau 1994), OEC redox states, non-photochemical quenching by PQ-pool, and PSII heterogeneity (see further details in Lazár and Schansker 2009, and references therein).

The addition of ultrafast photochemical processes to TEG models (Lazár and Pospíšil 1999; Lazár 2003; Lebedeva et al. 2000, 2002; Zhu et al. 2005; Belyaeva et al. 2006) opened the possibility of calculating the fluorescence yield not as a function of the actual concentration of the fluorescence quenchers (e.g., Q_A, $P680^+$, oxidized PQ in PQ-pool), but as a function of radiative deactivation of Chl *a* excited states, [ES] ($\Phi(t) = (k_F/k_L)\cdot[ES](t)$, where k_F is the rate constant of prompt Chl *a* fluorescence emission, k_L

is the rate constant of light excitation, and Chl *a* excited state [ES] is formed either in PSII RC or in the light harvesting antenna; see Belyaeva et al. (2006, 2008, 2011). On the other hand, it is important to note that the proper use of this relationship requires a correct estimation of [ES](t), which is dependent upon the chosen model. However, the details of processes related to energy transfer, trapping, and charge stabilization in PSII units are still under study, while the validity of the RRP models of the ultrafast PSII photochemistry (often used in the simulation of fast FI) has lately been questioned (Van der Weij-de Wit et al. 2011).

All the results obtained in the simulation of the O–J–I–P transient in dark adapted samples with these TEG models show that Q_A is progressively reduced during the fluorescence rise from the O to the P level, while the PQ-pool is reduced gradually from the J to the P level. As we have stated earlier, the functional assignment for the O–J and the J–I–P kinetic phases under conditions of saturating light is debatable and still elusive. In a number of alternative theories, Q_A is assumed to be in fully reduced form in all the active PSII centers, at the end of the O–J phase. This latter idea was based on the fact that the photoreduction of Q_A in high light is much faster than the forward electron transfer from the reduced Q_A to Q_B and the binding of PQ at the Q_B pocket (e.g., $\tau_{1/2}$ = 200–400 µs for Q_B reduction, Crofts and Wraight 1983; and $\tau_{1/2} \approx$ 1–2 ms for Q_BH_2/PQ exchange, Crofts et al. 1984).

However, mathematical simulations of the fast FI, even with the simplest TEG models, in which the apparent rate constant of Q_A reduction, k_L, has values corresponding to conditions of saturating light used in commercial fluorometers, does not lead to theoretical simulated curves showing a complete reduction of Q_A at a time as early as few milliseconds (Stirbet and Strasser 1995, 1996; Lazár et al. 1997; Stirbet et al. 1998; Strasser and Stirbet 2001; Tomek et al. 2001; Lebedeva et al. 2002; Lazár 2003; Zhu et al. 2005), as was suggested for the first time by Delosme (1967).

Further, the fluorescence transients simulated with TEG model(s) show usually two intermediary inflections, which have been interpreted to represent the J and I steps that are present in measured O–J–I–P curves (Fig. 2). The first inflection (J) is situated generally at ~2 ms (in agreement with experimental data). In simulations using only PSII reactions, the second inflection "I" of the fast FI seems to be connected with Q_BH_2/PQ exchange reaction at the Q_B-site, as it disappears when its rate constant is considered zero (Stirbet and Strasser 1995). However, in these earlier studies, the position in time of the I-step, and the total rise time of simulated O–J–I–P curve were shorter than those measured experimentally. Their exact occurrence varied as a function of the specific TEG model used (e.g., from ~20 to 50 ms for the total rise time to F_M). Therefore, simulation of the thermal phase using models consisting of only PSII components, and a regular second order reaction at a fixed rate constant for the Q_BH_2/PQ exchange, seems to be unsatisfactory; thus, other mechanisms must be considered to slowdown even more the Q_BH_2/PQ exchange reaction, contrary to the expectations expressed by those arguing for the separation of the photochemical and thermal phases based on kinetic differences between Q_A and Q_B reduction, and Q_BH_2/PQ exchange at the Q_B site.

Different solutions have been offered in order to improve the simulation of the J–I–P phase (i.e., the thermal phase) with TEG models (see Lazár and Jablonsky (2009) for a methodological approach in mathematical simulation of the O–J–I–P transient). We list below several of these approaches:

- Use of a rate constant for the Q_BH_2/PQ exchange reaction that decreases exponentially during illumination (Renger and Schulze 1985).
- Inclusion of PSII heterogeneity of both the antenna and the PQ-pool size (Renger and Schulze 1985; Hsu 1992).
- Incorporation of the effect of ΔpH and membrane potential (ΔΨ) changes, during illumination, on the rate constants of electron transfer reactions between Q_A and Q_B (Robinson and Crofts 1984), and on the Q_BH_2/PQ exchange (Riznichenko et al. 1999, 2000; Lebedeva et al. 2000, 2002; Belyaeva et al. 2003, 2008).
- Addition of ultrafast reactions taking place at the RC level (Baake and Schlöder 1992; Lazár 1999, 2003; Lebedeva et al. 2000, 2002; Zhu et al. 2005).
- Addition of other reactions besides those taking place in PSII (Baake and Schlöder 1992; Riznichenko et al. 1999, 2000, 2009; Lebedeva et al. 2002; Kroon and Thoms 2006; Laisk et al. 2006a, b; Rubin and Riznichenko 2009; Laisk et al. 2009a, b; Lazár 2009).

Baake and Schlöder (1992) were the first to consider adding, to the TEG model, reactions taking place beyond PSII; this was based on the findings of Munday and Govindjee (1969a) on the origin of the dip D of the OIDP transient. Baake and Schlöder (1992) attempted to simulate with their extended model this dip, but without success. Perhaps, this was because they had added only the PSI reactions in the reaction scheme, without considering explicitly also the Cyt b_6/f complex. Indeed, simultaneous measurements of the fast FI curve and 820 nm transmission signal (ΔA_{820}) (reflecting the redox state of the primary donor of PSI, P700 and plastocyanin, PC) (Schansker et al. 2005) revealed the importance of Cyt b_6/f and PSI during the thermal phase (i.e., the J–I–P rise).

Schansker et al. (2005) showed that in the presence of plastoquinone antagonist dibromothymoquinone (DBMIB), the fluorescence rise from the J level to the P level is much

faster than in its absence, so that P = I (i.e., the inflection at the I level disappears), and the fluorescence yield increases in a biphasic manner, as often observed in isolated chloroplasts (Bukhov et al. 2003; Srivastava et al. 1995). Since DBMIB inhibits the linear electron transport (LET) chain at the Cyt b_6/f level (Trebst et al. 1970; Böhme et al. 1971; Böhme and Cramer 1971; Kitajima and Butler 1975; Bowes and Crofts 1981; Yan et al. 2006; Cramer et al. 2006), only PSII reactions until PQ-pool would be responsible for the fluorescence rise. The suppression of the inflection at the I level by DBMIB supports the notion that, in TEG models, the mechanism of Q_BH_2/PQ exchange reaction must be treated differently (see the above list of different approaches).

Emphasizing that the inhibition by DBMIB in these experiments is limited only to Cyt b_6/f, Schansker et al. (2005) suggested that the inflection I is related to reactions taking place after the reduction of PQ-pool, being due to a transient bottleneck of the LET at PSI level, which influences the reoxidation of the reduced PQ-pool by Cyt b_6/f ($\tau_{1/2} = 20$ ms). Hence, in a sense, it can still be said that the second inflection of the fast FI curve (i.e., the I level) is due to the Q_BH_2/PQ exchange reaction, but as influenced by both PSII and PSI reactions, one reducing and the other oxidizing the PQ-pool, respectively. Based on this interpretation, the entire I–P phase of the fast FI would reflect the influence of both PSII and PSI activity on the fluorescence yield, the level I being due to a transitory equilibration of their respective kinetic influences on Q_A reduction, and the level P being the result of a transitory *traffic jam* in the LET chain, as first suggested by Munday and Govindjee (1969a, b).

The origin of the above-mentioned transitory blockage of LET was attributed to the inactivation of ferredoxin-$NADP^+$-reductase (FNR) during the dark period before measurement (Schansker et al. 2005). These conclusions were supported also by Lazár (2009), who had used a mathematical model simulating, in parallel, both the ΔA_{820} signal and the fast FI, as measured by Schansker et al. (2005) (see www.e-photosynthesis.org, where the mathematical model developed by Lazár is currently available; Safranek et al. 2011). Lazár added to a TEG model the reaction schemes for Cyt b_6/f complex (Cramer and Zhang 2006; see also Fig. 1), and PSI, with its acceptor side up to $NADP^+$. The main conclusions of Lazár (2009) were: (1) the redox state of Cyt b_6/f complex plays a major role in the kinetics of PQ-pool reduction; (2) plastocyanin (PC) is reduced during the dark period before illumination; (3) CET-PSI (PGR5) must be considered in the model, and its contribution to the shape of the FI curve is important, mainly at low light intensities; (4) the activation of FNR affects the I–P phase: this phase can be suppressed partially or totally by an active FNR, in a way similar to its suppression in samples treated with methylviologen (MV), which accepts electrons efficiently from PSI.

However, others have also included in their simulation of the fast FI mathematical models components of the entire LET chain, and have obtained results in agreement with the modified version of Duysens and Sweers Theory, supporting the idea that in order to reach the maximum fluorescence yield (F_M), *it is necessary*, *and sufficient* to have Q_A completely reduced in all the active PSII centers. For example, Rubin and coworkers (Riznichenko et al. 1999, 2000, 2009; Lebedeva et al. 2002; Rubin and Riznichenko 2009) developed a complex model that includes PSII, Cyt b_6/f, and PSI all the way up to the oxidized nicotinamide-adenine dinucleotide phosphate, $NADP^+$; further, they also included CET-PSI that was considered to be coupled with the generation of the proton motive force (pmf) across the thylakoid membrane (i.e., the one involving the so-called proton gradient regulator PGR5, and the electron transport from P700 to Fd, through FNR and Cyt b_6/f; Munekage et al. 2002, DalCorso et al. 2008), as well as ATP synthesis (see Fig. 1). The influence of $\Delta\Psi$(t) and ΔpH(t) during the fast FI was very important in their model, especially for the location of the inflection I and the kinetics of the I–P rise. Another model, which included, besides a simplified LET, alternative ET paths and major biochemical processes functioning in stroma and cytosol, was used by Laisk et al. (2006a, b) (see also Laisk et al. 2009a, b). Further, this model was successful in simulating also the signals correlated with the redox state of P700 and PC (Laisk et al. 2006a). Finally, we mention a model by Kroon and Thoms (2006), which includes PSII, PQ, Cyt b_6/f, PC, PSI, Fd, and CET-PSI; it simulates the FI in phytoplanktons. (We note that these authors have simulated the FI curves obtained by the FRR technique, not the O–J–I–P transient.)

Regarding the question posed in the title of "Mathematical simulation of the O–J–I–P transient: Is it possible to describe both the photochemical and thermal phases based on the modified version of Duysens and Sweers Theory?" section, our answer follows.

We conclude that studies using structure-based mathematical models in the simulation of the O–J–I–P transient have proven that *both photochemical and thermal phases can be described convincingly* (see e.g., Lazár 2009; Riznichenko et al. 2009; Rubin and Riznichenko 2009), and that, under saturating light conditions used in commercial fluorometers, Q_A is reduced completely only at the F_M level, even when some other quenchers or processes affecting the fluorescence yield are also considered, as we have assumed in the modified version of Duysens and Sweers Theory. Moreover, building of trans-thylakoidal ΔpH and $\Delta\Psi$, and electron transport reactions beyond PSII, related to PSI activity, have been shown to have an

important impact on the thermal phase of the O–J–I–P transient (confirming the conclusions reached by Munday and Govindjee 1969a, b, and by Schansker et al. 2005).

Indeed, all the results obtained through structure-based mathematical modeling discussed in this section have demonstrated that to simulate experimental O–J–I–P curves, there is no need to assume the existence of any hypothetical additional quencher (R or Q_2) that keeps the fluorescence low in the presence of closed PSII RCs and is removed during the thermal phase, as suggested by Delosme (1967) (or Joliot and Joliot 1977, 1979, 1981a, b).

Interpretation of the fluorescence induction O–J–I–P based on alternative theories

Delosme (1967) had suggested (cf. "Fast fluorescence induction: Early interpretations by L. N. M. Duysens, R. Delosme, and P. Joliot" section) that only ~50 % of the variable Chl *a* fluorescence is due to the photochemical closure of PSII RCs, and related to the redox state of Q_A, while the other ~50 % has a non-photochemical origin, and is related to the presence of a hypothetical secondary quencher R. Some of the arguments in favor of this type of theory listed by Schansker et al. (2011) are:

(1) The maximum fluorescence yield reached after a saturating STF (F_m^{STF}) is only 50–65 % of that measured in continuous saturating light (F_M) (Schreiber 1986; Samson and Bruce 1996; Steffen 2003; Steffen et al. 2005; Vredenberg et al. 2006). Moreover, the J level of the O–J–I–P transient measured at very high light, of 15,000 μmol photons m^{-2} s^{-1}, reaches an amplitude not higher than 60–65 % of F_M (see Fig. 6a, modified from Fig. 1 in Schansker et al. 2011).

(2) The I–P phase of the O–J–I–P transient is not eliminated by illumination with light intensity as high as 15,000 μmol photons m^{-2} s^{-1} (see Fig. 6a).

(3) The maximum fluorescence yield (F_M) is reached only when the entire electron transport chain is reduced (see e.g., Munday and Govindjee 1969a, b), while there are two rate limiting steps between Q_A and PSI, related to PQH_2/PQ exchange.

(4) The J and I steps do not change positions in response to changes in light intensity, as expected if they are due to rate limitations of PQH_2/PQ exchange (see the dashed lines in Fig. 6).

The argument (4), given above, stating that the steps J and I do not change positions in response to changes in light intensity, is untenable, since in agreement with the definitions of J and I (Strasser et al. 1995), these steps are positioned on the fluorescence maxima (or shoulders), not on the dips (see the arrows, cf. with the dashed lines in Fig. 6). It is clear from this figure that, with increasing light intensities, the J and I steps (Strasser et al. 1995) move toward shorter times (see Fig. 6a, b); moreover, with decreasing temperatures, the J and I steps move toward longer times (see Fig. 6c).

In the following sections, we examine critically the interpretation of the fast FI given by the alternative theories, in which it is assumed that in order to reach the maximum fluorescence yield (F_M) *it is necessary, but not sufficient* to have Q_A completely reduced in all the active PSII centers. We will discuss separately the photochemical

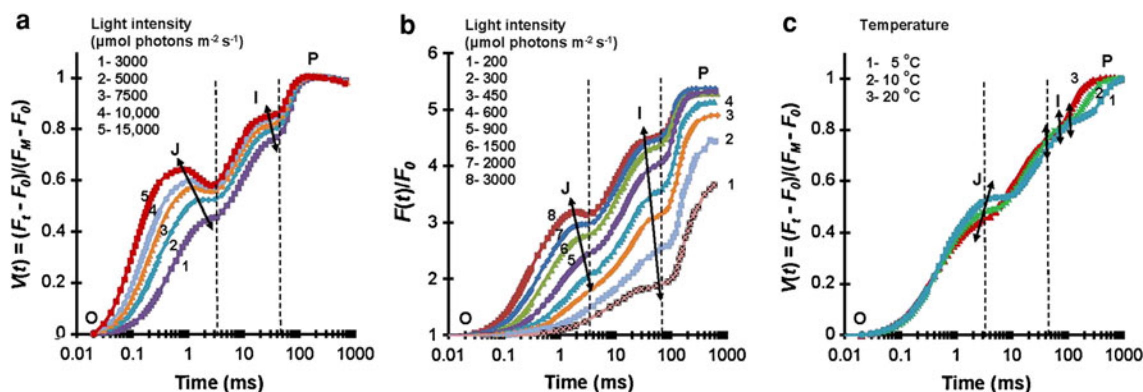


Fig. 6 The O–J–I–P chlorophyll fluorescence transients measured on pea leaves (**a**, **c**) or tobacco leaves (**b**). **a** Fast fluorescence transients measured at light intensities between 3,000-15,000 μmol photons m^{-2} s^{-1}. **b** Fast fluorescence transients measured at light intensities between 200-3,000 μmol photons m^{-2} s^{-1}. **c** Fast fluorescence transients measured at light intensity of 3,500 μmol photons m^{-2} s^{-1} at three different temperatures: 5, 10, and 20 °C. The O–J–I–P transients shown in the **a** and **c** were doubly normalized between F_0 and F_M, presented here as relative variable fluorescence $V(t) = (F(t) - F_0)/(F_M - F_0)$. The O–J–I–P transients shown in **b** were normalized at F_0. *Vertical dashed lines* show the location of the inflections J and I as indicated by Schansker et al. (2011). The *arrows* show the location of the inflections J and I as judged by AS and G, authors of this paper. Modified from Schansker et al. (2011)

phase (the O–J rise) and thermal phase (the J–I–P rise) of the fluorescence transient, and present explanations of some of these theories.

The photochemical phase of the fast Chl fluorescence induction (Q_A photoreduction)

As we have already pointed out, the photochemical and thermal phases of the fast FI are assumed to have different origins in the alternative theories. Thus, we ask if the thermal phase originates from a cause that is already present, and already acting, during the photochemical phase.

The reduction of Q_A during the O–J phase: are all PSIIs closed at the J level?

In the views of Delosme (1967), the O–J phase of the O–J–I–P transient measured under saturating continuous light conditions is assumed to reflect the completion of the photochemical phase, during which all Q_A molecules in the active PSII centers are reduced. Several arguments were given in favor of this idea: (a) relative temperature insensitivity of the O–J phase compared with the thermal phase, the J–I–P phase (Morin 1964; Delosme 1967; Schreiber and Neubauer 1987; Neubauer and Schreiber 1987); (b) close values of the maximum fluorescence yield measured after a saturating STF, and the fluorescence yield reached at the J level; and (c) the similarity between the rise time of the O–J phase, and that of the fluorescence transient measured in DCMU-treated samples (in which Q_A^- reoxidation is blocked, as DCMU occupies irreversibly the Q_B-pocket; Velthuys 1981).

In an attempt to determine experimentally if at the J step Q_A had been reduced only once, or 2–3 times, Schansker et al. (2011) used a multi-signal instrument (mPEA of Hansatech Instruments) to measure simultaneously the kinetics of the 40 µs component of the delayed fluorescence (also called delayed light emission, DLE), and the fast prompt fluorescence (see Fig. 7a, b). They found that at 5,000 µmol photons m^{-2} s^{-1} the DLE has a peak at 4 ms (see the dashed line in Fig. 7a), while at 1,150 µmol photons m^{-2} s^{-1}, the peak shifts towards 10 ms (Schansker et al. 2011); moreover, the 4.3-fold decrease of the light intensity led to a ~2.6-fold decrease of the maximum DLE amplitude. Further, Schansker et al. (2011) showed that the declining part of the DLE-signal is inversely proportional to the fluorescence rise during the rest of the thermal phase (see Fig. 7b).

The DLE induction curve has often been compared to the FI transient when placed on the same time scale (see early reviews: Govindjee and Papageorgiou 1971; Krause and Weis 1991; Malkin et al. 1994); it has similarities with the Kautsky curve of FI (Wraight and Crofts 1971; Satoh and Katoh 1983; Bilger and Schreiber 1990; Schmidt and Schneckenburger 1995; Goltsev et al. 2003, 2005, 2009), showing a rapid-rising peak, followed by a dip or a plateau, and a slow rise to a second transient maximum (see the DLE curve in Fig. 7a). The interpretation of DLE induction is not that straightforward, and therefore, we will present some background on the DLE signal.

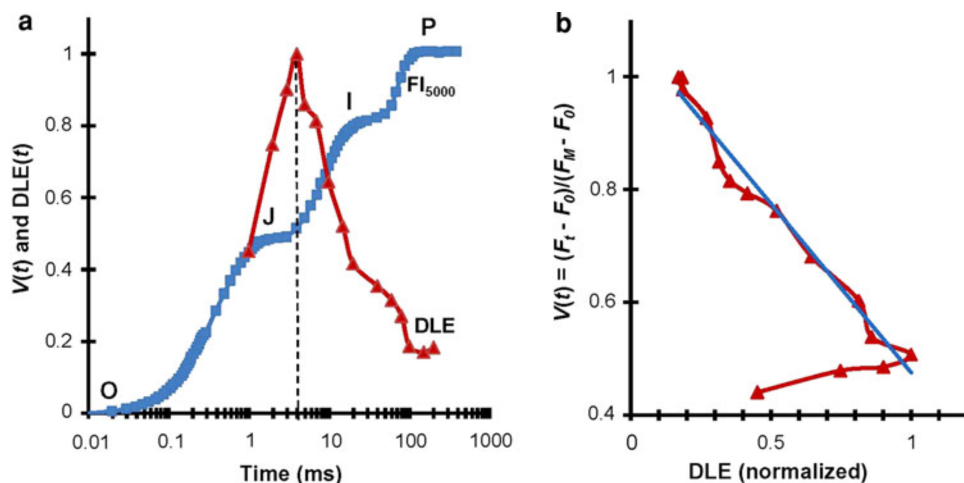


Fig. 7 **a** The O–J–I–P chlorophyll fluorescence and the Delayed Light Emission (DLE 40 µs) induction curves are shown; they were measured in parallel in pea leaves, at 5,000 µmol photons m^{-2} s^{-1}. The O–J–I–P transient was doubly normalized between F_0 and F_M, presented as relative variable fluorescence $V(t) = (F(t) - F_0)/(F_M - F_0)$. The DLE (*t*) induction curve was normalized at its maximum value. The *dashed line* marks the position in time of the DLE maximum. **b** The relationship between the DLE and *V* is shown. The linear trend between DLE and *V* (see the text in the ordinate of the figure), during the descending part of the DLE induction curve, is represented by a *straight line*. Modified from Schansker et al. (2011)

It is known that in oxygenic photosynthetic samples, DLE is generated mainly at the PSII level, via thermally activated recombination of separated charge pairs produced during forward electron transport (see reviews: Lavorel 1975; Govindjee and Jursinic 1979; Jursinic 1986; Tyystjärvi and Vass 2004; Goltsev et al. 2005, 2009). In contrast to the prompt fluorescence, which decays in darkness within 4–5 ns, the decay of DLE in dark can take even hours, and, further, it contains many kinetic components (Tyystjärvi and Vass 2004). The fastest component of the DLE is due to the radiative recombination between $P680^+$ and Phe^-, which occurs within 2–4 ns (Jursinic 1986); however, it seems that this ns DLE component cannot be separated from the prompt fluorescence (Schatz et al. 1988). Yet, due to the reversibility of electron transfer reactions, radiative recombinations can occur in darkness at different delay times, giving rise to various kinetic components of the DLE signal; thus, the chain of redox reactions within PSII controls both the prompt and delayed chlorophyll fluorescence (see a tentative scheme of the reactions involved in the prompt and the delayed fluorescence in Stirbet and Strasser 2001). Moreover, various other processes taking place during photosynthesis, as e.g., the trans-thylakoidal proton gradient (ΔpH) or the membrane potential (ΔΨ), can also affect the DLE (Wraight and Crofts 1971; Pospíšil and Dau 2002). In conclusion, the DLE induction curves reflect the kinetics of the light induced intrinsic development of radiative recombination reactions, with the accumulation of luminescence precursors, and the development of a trans-thylakoid proton gradient (ΔpH) and membrane potential (ΔΨ) that may induce changes in the luminescence quantum yield.

Schansker et al. (2011) discussed various aspects of DLE in the following terms:

– The 40 μs DLE -signal is due to the radiative recombination between Q_A^- and $P680^+$ (Christen et al. 1998; and Grabolle and Dau 2007).
– The initial rapid rise of the DLE (see DLE curve in Fig 7a) is related to the high turnover of Q_A photoreduction, and also to a gradual increase of PSIIs with OEC in the S_3 state. At the peak of the DLE curve (i.e., during the J–I fluorescence rise; see the dashed line in Fig. 7 a), a major fraction of the active PSIIs are assumed to be closed, and in the S_3 state. Indeed, Grabolle and Dau (2007) had assumed that PSIIs with OEC in the S_2 and S_3 states are responsible for a higher DLE than PSIIs with OEC in S_0 and S_1 states. Consistent with this hypothesis is the suggestion that the reduction of Y_Z^+ is slowed-down when the OEC is in S_2 and S_3 states; consequently, $P680^+$ reduction will proceed also with a slower rate, which will increase the number of luminescence precursors in the $P680^+PheQ_A^-$ state.
– The subsequent descending part of DLE induction curve (see DLE curve in Fig. 7 a) is related to a slowing-down of Q_A reduction turnover.

However, in our view, there are problems and concerns with the views of Schansker et al. (2011) because of the following.

– The assumption that a major fraction of PSII centers have the OEC in the S_3 state when the DLE signal attains its maximum is not supported by the experimental data since: (a) the S_0:S_1 ratio is generally known to be ∼25:∼75 in dark-adapted samples (Kok et al. 1970); and (b) under continuous illumination, the advancement of the redox states of OEC is not synchronized.
– Even if we would accept that the number of closed PSIIs in the S_3 state is highest when the DLE signal reaches its peak, there is no reason to believe that there is a major fraction of closed PSII centers under that condition.
– It is difficult to understand how the descending part of the DLE induction curve is due to a “slowing-down of the Q_A reduction turnover” that leads to a decrease in the number of DLE precursors, since at the same time almost all PSII centers are considered already closed.

Further, Goltsev et al. (2005, 2009), who have made parallel measurement on the fast FI and DLE induction, had arrived at different conclusions than Schansker et al. (2011). They have reported that:

– DLE rises rapidly due to the photochemical accumulation of luminescence precursors, as well as a non-photochemical enhancement of the DLE signal that is due to an increase of the membrane potential (ΔΨ) (related to a transient accumulation of $P700^+$; Wraight and Crofts 1971; Pospíšil and Dau 2002).
– The antiparallel relation (except for the initial DLE rise) between the DLE induction curve and the fast FI transient (see Fig. 7b), as observed in different experimental conditions, was considered to reflect inverse proportionality between the DLE-signal and the fraction of closed PSII (see also Itoh 1980). Thus, in agreement with this interpretation, a large part of the DLE induction curve is proportional to the fraction of open PSII, which continues to decrease until F_M is reached.

In view of the above discussion, we conclude that the results of Schansker et al. (2011) do not provide sufficient reasons for the conclusion that a major fraction of PSIIs are closed where DLE signal is maximum (i.e., during the J–I rise). On the contrary, their data seems to be consistent with the idea that all the active PSIIs are fully closed at the

F_M level only, which is in agreement with the modified version of the Duysens and Sweers Theory.

In the following section, we analyze different theories that have provided interpretations for the fluorescence transient obtained after a saturating STF (see also Samson et al. 1999), as many of them seem to support the concept, advocated by the alternative theories, that *it is necessary*, *but not sufficient* to have Q_A completely reduced in all active PSII centers at F_M.

The reduction of Q_A after a saturating single turnover flash (STF)

The FI curve after a STF does not show a thermal phase due to the very short duration of the pulse, the fluorescence rise being assigned only to Q_A reduction. Therefore, STF measurements have the advantage of allowing us to have a more straightforward interpretation than the FI obtained with continuous multi-turnover light. These experiments have shown that the fluorescence maximum F_m^{STF} reached after a saturating STF is only 50–65 % of that measured in continuous light, F_M (Govindjee and Jursinic 1979; Schreiber 1986; Samson and Bruce 1996).

Several reasons for the low fluorescence yield observed after a saturating STF have been proposed. These include:

(1) Singlet–singlet annihilation process (Mauzerall 1978; Zankel 1973).
(2) Generation of P680 triplet ^{3}P680, which has a lifetime of hundreds of µs and is a strong quencher of the first singlet excited state (Barzda et al. 2000).
(3) Generation of long-lived quenchers (lifetimes longer than a few ms), originating probably from Chl cations, or other radicals that are produced either directly from Chl triplets, or via the singlet oxygen generated in reactions sensitized by the Chl triplet (Barzda et al. 2000).
(4) Presence of a fraction of PSIIs with oxidized NHI (that re-oxidize very fast some of the reduced Q_A), direct P680$^+$ quenching, and partial reopening of RCs by rapid charge recombination between P680$^+$ and Q_A^- (Boussac et al. 2011; Lavergne and Rappaport 1998).
(5) Donor-side quenching involving the redox states of OEC (Delosme 1971, Delosme and Joliot 2002; Srivastava et al. 1999; Jablonsky and Lazár 2008, Jablonsky et al. 2008).
(6) Quenching by the oxidized PQ-pool (Vernotte et al. 1979; Kramer et al. 1995; Prasil et al. 1996; Samson and Bruce 1996; Samson et al. 1999; Kurreck et al. 2000; Koblizek et al. 2001; Pospíšil and Dau 2002).
(7) Quenching by the oxidized PQ occupying the Q_B pocket (Samson and Bruce 1996; Vasilev and Bruce 1998; Kolber et al. 1998; Yaakoubd et al. 2002; Schreiber 2002).
(8) Quenching by oxidized Phe, and the absence of enhancement of the fluorescence yield by the membrane potential ($\Delta\Psi$) during the I–P phase, as a result of CET-PSI (Vredenberg 2000, 2011).
(9) A 2 times decrease of the rate constant of primary charge separation (P680*Phe → P680$^+$Phe$^-$) in closed RCs relative to open RCs, and a rapid non-radiative charge recombination between P680$^+$ and Phe$^-$ in closed RCs with an oxidized PQ in the Q_B-pocket (Schreiber and Neubauer 1989; Schreiber and Krieger 1996; Kolber et al. 1998; Belyaeva et al. 2006, 2008, 2011).
(10) Conformational change, enhancing the fluorescence yield, that occurs only when Q_A re-oxidation is blocked (e.g., Schansker et al. 2011).

We provide below a critical examination of these factors. Further, we will discuss their potential to act as quenchers of the fluorescence at the J level, and their possible influence on the thermal J–I–P phase, since a ratio of F_m^{STF} to $F_M < 1$ has often been used as an argument in favor of alternative theories.

The first three factors in the above list (i.e., singlet–singlet annihilation process, the generation of ^{3}P680, and of other long-lived quenchers) are known to decrease F_m^{STF} in proportion to the intensity of the flash, but for flashes with duration longer than picoseconds this decrease is too low to explain a value of $F_m^{STF}/F_M < 1$ observed experimentally for flashes of different intensities. On the other hand, the presence of a fraction of PSII with oxidized NHI, accumulated in samples kept for some time in the dark, can explain a lower fluorescence yield after a saturating STF, especially in DCMU-treated samples (Boussac et al. 2011). In addition, a direct P680$^+$ quenching, and partial reopening of RCs by rapid charge recombination of P680$^+$ with Q_A^-, proposed by Lavergne and Rappaport (1998), will also lower the maximum fluorescence. However, if we assume that all the active PSIIs are closed at the end of the photochemical phase of the fast FI measured in continuous saturating light (Delosme 1967), the above arguments cannot explain a decrease of up to ~50 % of the fluorescence yield at the J level.

On the donor side of PSII, the redox states of OEC have been shown to influence the maximum fluorescence yield reached after a saturating STF, but the variations observed were small, ~15 % (Delosme 1971; Delosme and Joliot 2002). Further, on the acceptor side, the de-quenching induced by PQ-pool reduction (Vernotte et al. 1979) cannot be the only explanation, because its amplitude is variable, and generally too small to generate the entire thermal phase (Krause and Weis 1991; Schreiber and Krieger 1996).

However, Pospíšil and Dau (2002) presented a compromise alternative model, in which the photogeneration of a positive membrane potential (ΔΨ) (i.e., with the positive side toward the lumen) was suggested to induce a fluorescence stimulation during the J–I rise, and PQ-pool quenching release, that would lead to fluorescence increase during the I–P rise. They explained the fluorescence stimulation by ΔΨ by assuming a decrease in the rate of primary radical pair generation, and an increase in the rate of charge recombination between $P680^+$ and Phe^-, both leading to the accumulation of excited states in closed PSII centers, and therefore to an increase of fluorescence emission (Dau and Sauer 1991, 1992). Yet, Tóth et al. (2005) have shown that the maximum fluorescence yield measured in continuous light in DCMU-treated leaves is not affected by the redox state of PQ-pool, and therefore, it seems that only in samples in which the membrane structure of the photosynthetic apparatus is damaged the oxidized PQ molecules can quench the fluorescence (Haldimann and Tsimilli-Michael 2005). Therefore, all the theories advocating the idea that the fluorescence dequenching (enhancement) due to PQ-pool reduction is at the origin of the thermal phase are contradicted by these results on intact leaves.

On the other hand, the results presented by Tóth et al. (2005) do not contradict the alternative theories suggesting that the oxidized PQ occupying the Q_B pocket quenches the fluorescence, both after a saturating STF and during the O–J–I–P transient (Samson and Bruce 1996; Vasilev and Bruce 1998; Kolber et al. 1998; Yaakoubd et al. 2002; Schreiber 2002). The assumed role played by Q_B as a quencher varies in these theories: from static quenching (Kurreck et al. 2000), to its control of the nonradiative charge recombination between $P680^+$ and Phe^- (Schreiber and Krieger 1996; see below for details), or direct participation in primary photochemistry via the inactive branch of PSII RC (Schreiber 2002). However, in our opinion, the idea of Q_B acting as a direct (or indirect) quencher advocated by these theories is not yet adequately supported experimentally.

In an original alternative theory, Vredenberg (2000, 2004, 2011) has added two more factors, other than the photochemical reduction of Q_A, that influence the fluorescence yield during the O–J–I–P transient: (1) oxidized Phe, acting as a quencher, and (2) membrane potential (ΔΨ) created during the I–P rise by CET-PSI, inducing fluorescence enhancement. Thus, in Vredenberg's view, the maximum fluorescence F_m^{STF} is lower than F_M, even if all active PSIIs are closed, because Phe is still mainly oxidized and CET-PSI is not functioning. However, Vredenberg's model has been received, in general, with skepticism, due to its controversial assumptions.

Another idea is that of a conformational change affecting the J–I–P rise (Moise and Moya 2004a; Schansker et al. 2011); this idea is more complex, and it will be discussed in details later in relation to the thermal phase of the fast FI. However, we will first present here a theory proposed by Schreiber and Krieger (1996), which assumes that the nonradiative charge recombination between $P680^+$ and Phe^- in closed PSII centers is the main cause of fluorescence quenching after a saturating STF.

The theory of Schreiber and Krieger (1996) can be explained using the RRP model of PSII photochemistry (Schatz et al. 1988; Leibl et al. 1989; Trissl et al. 1993; see also Dau 1994). The main ideas included in this model, in its most simplified form, are (see the reaction scheme of the RRP model in Fig. 8):

(1) Rapid exciton equilibration occurs between all the PSII pigments, including P680 (these are assumed to form a single pool, a multimer of pigments).
(2) The primary charge separation leading to the radical pair $[P680^+Phe^-]$ formation is reversible, and this reversibility influences fluorescence decay kinetics.

As shown in Fig. 8, the fluorescence yield can be affected by parameters that influence the yield of singlet excitation in PSII (both antenna and RCs). Within the

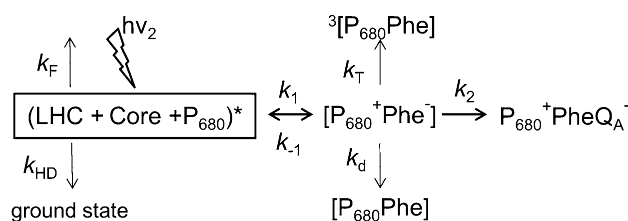


Fig. 8 A simplified scheme of the reversible radical pair (RRP) model for PSII photochemistry. All pigments connected with the photosystem II (PSII) (LHC + Core + P680) are assumed to form a single pool, and the primary charge separation is presumed to be reversible. LHC, light harvesting complex, Core represents the pigments of the core reaction center complex of PSII; P680 is the primary electron donor of PSII; Phe is pheophytin, the primary electron acceptor of PSII; Q_A is the primary plastoquinone acceptor of PSII; k_F is the rate constant of the radiative energy dissipation at the antenna level (fluorescence emission, delayed light emission); k_{HD} is the rate constant of nonradiative energy dissipation at the antenna level (internal conversion, quenching by triplet states, energy spillover to PSI or transfer to another PSII exogenous fluorescence quenchers); k_1 is the rate constant related to the intrinsic rate constant of primary charge separation; k_{-1} is the rate constant of radiative charge recombination that leads to re-excitation of the antenna and DLE; k_2 is the rate constant of the decay of radical pair by electron transfer to Q_A; k_T is the rate constant of the decay of the radical pair through ^3Chl generation; k_d is the rate constant of the decay of the radical pair through nonradiative recombination to the ground state. Modified from Dau (1994) (*Note*: The rate constants k_1, k_{-1}, and k_d have different values for the open than for closed centers; see e.g., Fig. 5.3 in Lazár and Schansker 2009)

framework of the RRP model, and knowing that the fluorescence yield is substantially increased when Q_A is reduced, the fluorescence increase is attributed to changes at the RC due to the local electric field generated by Q_A^-: a decrease of the rate constant of primary charge separation k_1, and eventually an increase of the radiative recombination rate (k_{-1}) after closure of PS RCs (Schatz et al. 1988; Leibl et al. 1989; Trissl et al. 1993; Gibasiewicz et al. 2001). These changes lead to an increase in the number of excited states that contribute to the variable fluorescence, $F_V(t) = F(t) - F_0$.

The RRP model was used with success to simulate the fluorescence rise in the presence of DCMU (Lavergne and Trissl 1995; Trissl and Lavergne 1995; Lazár and Pospíšil 1999), as well as the O–J–I–P transient, when included into an extended TEG model (Lazár 2003; Lebedeva et al. 2000, 2002; Zhu et al. 2005; Belyaeva et al. 2006); in these simulations an $\sim$6 times decrease of the rate constant of primary charge separation (k_1) after the reduction of Q_A was assumed (see Fig. 8). Based on the findings of Renger et al. (1995), Schreiber and Krieger (1996) state that this decrease is only moderate, $\sim$2 times. In this case, the RRP model predicts that, besides direct fluorescence, which is increased after Q_A reduction, delayed fluorescence originating from the primary radical pair recombination (ns DLE) would contribute to the F_V. This idea is similar to the original Klimov–Shuvalov hypothesis (Shuvalov and Klimov 1976; Klimov et al. 1977; Shuvalov et al. 1980; Klimov 2003), in which, however, only ns DLE is assumed to contribute to the F_V, since the decrease of k_1 after Q_A reduction is not accepted. This hypothesis (the Klimov–Shuvalov hypothesis) is not supported by experimental data (see a discussion in Dau 1994).

Further, Schreiber and Krieger (1996) suggested that, during the photochemical phase, this ns DLE is completely quenched by nonradiative charge recombination of the primary radical pair [$P680^+Phe^-$], while at the end of this phase all the active PSII centers are closed (Delosme 1967). However, during the thermal phase, the rate constant of this energy dissipative reaction is supposed to gradually decrease due to unspecified factors, eventually correlated with the Q_B-site occupancy.

A major issue regarding the above theory is related to the concept that ns DLE contributes significantly to the variable fluorescence, which, by itself, is controversial. Under these conditions, the question is whether an increase in ns DLE during the thermal phase can account for 30–50 % of the variable fluorescence. We note that the possibility of a considerable contribution of ns DLE to the variable fluorescence is not predicted by RRP models, in which the decrease of the quantum yield of primary charge separation in closed PSII centers is assumed to be high (Schatz et al. 1988; Roelofs and Holzwarth 1990, Trissl 2002). Moreover, Samson et al. (1999) suggest that decay kinetics of the fluorescence yield induced by a STF could not be satisfactorily described by a low yield of ns DLE during the photochemical phase, as proposed by Schreiber and Krieger (1996). Samson et al. (1999) consider, instead, that the low fluorescence yield after the STF is due to a non-photochemical quenching process at the antenna level, induced by oxidized PQ molecules in the PQ-pool; they based their conclusion on experiments with isolated thylakoids suspended in the presence of the artificial antenna quencher, 5-hydroxy-l,4-naphthoquinone. However, this explanation cannot be sustained since the PQ-pool quenching theory has been challenged by the experiments of Tóth et al. (2005), at least in intact leaves (see above).

The theory of Schreiber and Krieger (1996) (i.e., nonradiative charge recombination between $P680^+$ and Phe^- in closed PSII centers as the main cause of fluorescence quenching) is difficult to be incorporated into a structure-based mathematical model that would simulate the O–J–I–P curves, since the processes responsible for changes in the kinetics of energy dissipative reactions during the J–I–P thermal phase had not been precisely considered by these authors. However, we will discuss in the next section the application of the idea of Schreiber and Krieger (1996) in the simulation of the fluorescence transient induced by a saturating STF (Belyaeva et al. 2006, 2008, 2011).

Numerical simulation of the fluorescence transient induced by a saturating STF: is the rate of nonradiative recombination between $P680^+$ and Phe^- the key parameter for a lower F_m^{STF} value than F_M?

The STF method is very useful for the study of ultrafast PSII reactions of excitation energy transfer, charge separation, and stabilization (see an early review: Govindjee and Jursinic 1979). The time dependence of the fluorescence yield depends on several processes including excitation energy transfer in the antenna complexes (fs–ps time domain) (Schatz et al. 1987, 1988; Renger and Holzwarth 2005; Clegg et al. 2010), and electron transfer reactions (ps–ms) (Crofts and Wraight 1983; Eaton-Rye and Govindjee 1988a, b; Leibl et al. 1989; Steffen et al. 2005; Kern and Renger 2007). Since the reduction of Q_A after a saturating STF should be completed within 1 ns (Nuijs et al. 1986; Eckert et al. 1988), the maximum fluorescence yield should be observed instantaneously after the application of the actinic flash. Instead, it was found that the fluorescence yield reaches its maximum F_m^{STF} only 20–50 μs after the flash.

A significant delay in the fluorescence rise after a saturating STF was first observed by Mauzerall (1972) who assigned its origin to structural changes in the RC induced by the actinic flash. Butler (1972) interpreted it to be due to

the time taken for the reduction of the quencher $P680^+$ (also see Shinkarev and Govindjee 1993); further, carotene triplet (^{3}Car) has also been suggested to be another possible quencher of fluorescence (Duysens et al. 1972, 1975; Zankel 1973; Mauzerall 1976). In addition, Zankel (1973) had earlier considered Y_Z^+ to act as a quencher of Chl *a* fluorescence during this period of time. Moreover, we note that Vredenberg (2009) assumed quenching by Y_Z^+ not only to account for the attenuation of F_m^{STF} in sub-µs light flashes, but also to explain the sigmoidicity of the initial O–J rise in actinic light of moderate intensities.

Steffen (2003) and Steffen et al. (2001, 2005), using an equipment monitoring fluorescence rise starting at 100 ns after an actinic flash from a frequency-doubled Nd-YAG laser ($\lambda = 532$ nm and fwhm = 10 ns), have followed its subsequent decay during 10 s (see Steffen et al. 2001 for details). Steffen et al. (2005) were convinced that, contrary to the common belief, the quenching by $P680^+$ is higher by a factor of two in comparison to the quenching by Q_A. Using specific rate constants for the efficiency of non-photochemical quenching and additional dissipative processes, these authors were able to calculate, based on a so-called '3-quencher' model, changes in [$P680^+$], [Q_A], and [^{3}Car] with time (*t*). In the experiment with the highest laser flash intensity used (7.5×10^{16} photons/cm^2 per flash), the initial drop of the fluorescence yield following the excitation was explained mainly by ^{3}Car quenching, since the rapid decline of fluorescence below the F_0 value disappears with a typical time constant of ^{3}Car lifetime of ~5 µs (Steffen et al. 2005; see also Duysens et al. 1975; Mauzerall 1976).

Simulation of the fluorescence induction after a 10-ns single turnover flash by Andrew Rubin and coworkers

In order to obtain detailed information, and to verify the results of Steffen and collaborators, Belyaeva et al. (2006, 2008, 2011), in the research group of Rubin, used an extended TEG model, originally developed for the analysis of the O–J–I–P transient, which included the '3-quencher' hypothesis proposed by Steffen et al. (2001, 2005), mentioned above. Belyaeva et al. (2006, 2008) were able to simulate and fit the time dependence of the fluorescence yield measured in different experiments on the green alga *Chlorella pyrenoidosa*, with and without DCMU. Belyaeva et al. (2011) extended this work to leaves of *Arabidopsis thaliana* exposed to different laser pulse intensities. Their mathematical model includes 28 different redox states of PSII, plus PQ and PQH_2 (see the reaction scheme in Belyaeva et al. 2006, 2008, 2011).

The model of Belyaeva and coworkers has the following features:

(1) The reaction scheme explicitly includes ⟨Chl (antenna)-P680⟩ (i.e., the primary PSII donor P680 in equilibrium with Chl antenna), Phe, Q_A, Q_B, and PQ-pool. However, Y_Z, and the cycling of the S-states (i.e., that lead to reduction of $P680^+$) are only indirectly considered.

(2) A RRP model of the photochemistry taking place at the PSII RC level (Schatz and Holzwarth 1986; see Fig. 8) is used; it includes: (a) trapping of the excitation energy; (b) primary charge separation, with the rate constant of forward and back reactions being influenced electrostatically by the negative charge on reduced Q_A (however, only moderately; Renger et al. 1995), and also by transmembrane $\Delta\Psi$ and ΔpH (Schatz et al. 1988; Meiburg et al. 1983; Keuper and Sauer 1989); and (c) different energy dissipation reactions, which allow verification of the theory of Schreiber and Krieger (1996).

(3) The '3-quencher' model of Steffen (2003) and Steffen et al. (2001, 2005) is accepted, which considers, in addition to the photochemical quenching via [Q_A], the non-photochemical quenching by [$P680^+$] and [^{3}Car].

In the model of Belyaeva and coworkers, the time-dependent evolution of the fluorescence yield was calculated by multiplying the fraction of all fluorescent PSII states (⟨Chl-P680⟩*) by k_F/k_L, where k_F is the rate constant of fluorescence, and k_L is the rate constant for excitation with light, which were assumed to be independent of the redox states of Q_A and Q_B, or the occupancy of Q_B site.

Belyaeva et al. (2008) assumed that the strong actinic illumination transfers a very high proportion of the RCs (~91 %) into a sort of 'metastable' state [$P680^+PheQ_A^-$] at $t = 100$ ns after the laser pulse (Steffen et al. 2001, 2005; Steffen 2003), and they used it as an initial value for the simulation of FI starting at $t = 100$ ns; later, Belyaeva et al. (2011), using data obtained at different intensities of the STF, simulated the PSII redox states generated by the actinic laser flashes. Belyaeva et al. (2011) considered that, for all intensities of the laser flash, the parameters for charge separation, stabilization, and Q_B-site reactions remain constant; however, the parameters of dissipative processes in the antenna (i.e., heat dissipation and ^{3}Car quenching), and the rate constant of charge recombination reaction between $P680^+$ and Phe^- had to be reduced for decreasing values of the laser flash energy. In our view, there are several possible reasons for the necessity to adjust these parameters as function of STF intensity, which had not been taken into account in the model. These are:

– Existence of a fraction of PSIIs with oxidized NHI (see Boussac et al. 2011).
– Photogeneration of long-lived fluorescence quenchers, other than ^{3}Car (see "Modulation of the fast FI by

processes or components of the photosynthetic apparatus other than Q_A" section).

– Influence of processes, not yet specified, taking place at high light that lead to changes in rate constants of the primary photochemistry. The increase of F_m^{STF}/F_0 from ~2 to ~3, when the duration of the saturating STF varies from ns to μs, seems to be in favor of the photogeneration of unspecified quenchers by high light pulses, and/or the implication of unknown mechanisms inducing antenna quenching, or changing the primary photochemistry.

Belyaeva et al. (2006, 2008, 2011) have confirmed the results of Steffen et al. (2001, 2005; Steffen 2003). Moreover, they have added new information on the importance of specific PSII electron transport reactions, radiative and nonradiative charge recombinations, and several dissipative processes taking place during the fluorescence rise and decay after a 10-ns STF laser pulse. Their mathematical simulations show that laser pulses induce a rapid (1 ns) increase in the fraction of closed PSII centers, $[Q_A^-]$, to a saturating value (or close to it, as function of the intensity of the pulse), which is maintained almost at a constant value during the fluorescence rise to its maximum F_m^{STF}: i.e., $[Q_A^-] = 100$, 95, and 88 % for 100, 8.3, and 4 % of maximum laser flash intensity (Belyaeva et al. 2011), respectively. However, the simulated fluorescence increase until F_m^{STF} was shown to be critically dependent on non-photochemical quenching by $P680^+$ and ^{3}Car, the first prolonging its influence to some extent until the fluorescence reached its maximum. Further, the simulations also revealed that the slope of fluorescence rise, and the rise time (t_m) necessary to reach F_m^{STF}, depend(s) highly on the rate constant values of $P680^+$ oxidation by Y_Z (confirming the data reported by Den Haan et al.1974; Jursinic and Govindjee 1977), and non-photochemical quenching by carotenoid triplets (Duysens et al. 1975; Mauzerall 1976).

In summary, the results obtained by Belyaeva et al. (2006, 2008, 2011) show that, theoretically, it is possible to simulate the FI curve measured after a 10 ns STF that have F_m^{STF}/F_0 of 1.8, as observed experimentally. The measured curves were fitted well with a model that used rate constants of 5.5×10^8 and 4×10^7 s^{-1} for nonradiative and radiative recombination of $[P680^+Phe^-]$ in closed RCs. Also, the ratio of the rate constants of primary charge separation in open (k_1^{ox}), and closed (k_1^{red}) RCs, was: $k_1^{ox}/k_1^{red} \sim 2$ (Belyaeva et al. 2006, 2008, 2011). Moreover, Belyaeva et al. (2011) concluded that the nonradiative charge recombination between $P680^+$ and Q_A^- also diminished the fluorescence intensity, but the simulations indicated that it affects mainly the fluorescence decay phase of the transient. We note, however, that in this model, the recombination between the S_2 state of OEC with Q_A^- and Q_B^- have been neglected, even when it is known that they influence the fluorescence decay during 10 s time domain simulated in this study.

The answer to our initial question: "Is the rate of nonradiative recombination between $P680^+$ and Phe^- the key parameter for a lower F_m^{STF} value than F_M?" *is complex.* In PSII centers, several rate constants affect the fluorescence yield (see Fig. 8):

(1) k_{HD}, the rate constant of nonradiative energy dissipation at the antenna level (important for the so-called 'antenna quenching').
(2) k_1, the rate constant of primary charge separation, $[P680^*Phe] \leftrightarrow [P680^+Phe^-]$.
(3) k_{-1}, the rate constant of radiative recombination between $P680^+$ and Phe^-.
(4) k_d, the rate constant of nonradiative recombination between $P680^+$ and Phe^-.

The initial fluorescence F_0 (defined as the fluorescence yield when all Q_A molecules are oxidized) will depend on the values of these rate constants for open PSII centers. In the RRP model, it is assumed that these rate constants change when PSII centers are closed (due to the influence of the negative electric charge of reduced Q_A; Gibasiewicz et al. 2001), so that the maximum fluorescence yield after PSII closure becomes $F_{max} > F_0$. We emphasize that both the calculated values of F_0 and F_{max} must fit the experimental data for a successful simulation of the FI curve.

Assuming that the lower value of F_m^{STF} compared to that of F_M is due to RC quenching, not antenna quenching (Schreiber and Krieger 1996; Belyaeva et al. 2006, 2008, 2011), the ratio F_m^{STF}/F_0 would depend on the way the rate constants of the reactions at RC level change after PSII closure. The results obtained by Belyaeva et al. (2006, 2008, 2011) indicate that, after Q_A reduction, a moderate decrease of k_1 (~2), a moderate increase of k_{-1} (~1.8), and a large increase of the ratio k_d/k_{-1} (≥7) (see Fig. 8 for the definitions of these rate constants in the RRP model) would lead to a low F_m^{STF}/F_0 ratio (i.e., ~2), as predicted by the theory of Schreiber and Krieger (1996). On the other hand, in agreement with Schreiber and Krieger (1996), in the case of O–J–I–P transient, all active PSII centers are closed at the end of the photochemical O–J phase, and the rate constants of the reactions at RC level during the O–J rise should be similar to those assumed after a saturating STF. However, for the thermal J–I–P phase, Schreiber and Krieger (1996) do not discuss the processes controlling the gradual decrease of k_d, until the fluorescence reaches a maximum F_M corresponding to a high ratio $F_M/F_0 \sim 5$–6.

Alternatively, if it is assumed that, in the presence of reduced Q_A, k_1 is drastically decreased (by ~6 times), k_{-1} remains unchanged, the ratio k_d/k_{-1} would moderately

increase ($\sim$2 times) (Schatz et al. 1988; Trissl et al. 1993; Trissl 2002) then, the simulated fast FI rise gives a high ratio of $\sim$6 for F_M/F_0, just as is observed in the experimental curve measured under saturating multi-turnover continuous light (Lavergne and Trissl 1995; Trissl and Lavergne 1995; Lazár 1999, 2003; Lebedeva et al. 2000, 2002; Zhu et al. 2005). In this case, the simulations are in agreement with the theory of Duysens and Sweers in its modified version, *and there is no necessity to assume the variation of any of these rate constants during the thermal J–I–P rise*. On the other hand, in this case, in order to explain the lower experimental value of F_m^{STF} compared to that of F_M, we may accept the hypothesis that extremely high light conditions produce structural changes that either modify the rate constants at the RC level as described above by Belyaeva et al. (2011), or increase energy dissipation reactions at the antenna. However, as we have shown in "The reduction of Q_A after a saturating single turnover flash (STF)" section, there are other mechanisms that could very well be responsible, in principle, for the lower F_m^{STF} than F_M.

The fast fluorescence transient of DCMU-treated samples: does the fluorescence induction in the presence of DCMU exhibit a thermal phase?

At saturating light intensities, the fluorescence transient of samples treated with DCMU, a known inhibitor of electron transport, consists of a fast rise phase (usually sigmoidal), with a rise time that is almost identical to the rise time of the J level in the O–J–I–P transient of uninhibited samples. DCMU and several other compounds are known to replace irreversibly Q_B from its pocket, blocking the reoxidation of reduced Q_A and interrupting the connection between PSII and PSI (Velthuys 1981; Vermaas et al. 1984). The FI curves measured in the presence of DCMU are simpler and, thus, easier to be described theoretically than the O–J–I–P curves, although there is no consensus on the interpretation for the origin of sigmoidicity in the DCMU curve (Joliot and Joliot 1964; Strasser 1978, 1981; Vredenberg 2000, 2008b; Schansker et al. 2011). Further, questions remain regarding the multi-exponential fluorescence kinetics as being due to PSII heterogeneity (Melis and Homann 1975, 1976; Lazár and Pospíšil 1999), or other causes, which will not be discussed here.

In the majority of alternative interpretations of the O–J–I–P transient it is assumed that in DCMU transients the fluorescence rise represents only the photochemical phase (Delosme 1967; Etienne and Lavergne 1972; Neubauer and Schreiber 1987). However, others suggest that processes similar to those responsible for the thermal J–I–P phase also influence the FI of DCMU-treated samples (see Joliot and Joliot 1977, 1979, 1981a, b; Vredenberg 2000, Vredenberg et al. 2006; Schansker et al. 2011).

We have already discussed the second quencher hypothesis of Joliot and Joliot (1977, 1979, 1981a), in which Q_2 is supposed to influence the fluorescence rise at the end of the transient. However, Vredenberg (2000) advocates that the oxidized Phe acts as a second quencher besides Q_A, and in addition, the membrane potential induced by CET-PSI ($\Delta\Psi$) leads to fluorescence enhancement. In his opinion, these two factors will influence the DCMU induction curve in addition to Q_A reduction. On the other hand, Schansker et al. (2011) observed that at room temperature, the rise time of the fluorescence transient curve, in the presence of DCMU, was longer than that of the O–J phase in untreated sample, even when the initial fluorescence rise kinetics during the first 100–150 μs of illumination were the same in both untreated and DCMU-treated samples (Pea leaves). Schansker et al. (2011) have assumed that during the fluorescence rise in DCMU-treated sample, every Q_A in PSII active centers is reduced only once until F_M, but that, in the untreated sample, a fraction of Q_A is reduced a second time until the J level is reached (Stirbet et al. 1998; Zhu et al. 2005). Therefore, Schansker et al. (2011) have concluded that the fluorescence rise in the presence of DCMU involves, besides the reduction of Q_A, also the induction of a process that is associated with the thermal phase. We note that this explanation is based on the preconception that at the end of the O–J rise, a major fraction of Q_A molecules in the active PSII centers are reduced, which is not the case, as we have discussed earlier. To us, these observations can be best explained by suggesting that at the J level only a fraction of PSII centers are closed (see Stirbet et al. 1998; Zhu et al. 2005). Further, Schansker et al. (2011) reported experimental data on FI in DCMU-treated samples obtained at low temperatures, which were interpreted to indicate the existence of a thermal phase originating from a conformational change. We will discuss these results by Schansker et al. (2011) in a separate section later, when we will present also their theory involving a conformational change taking place during the O–J–I–P transient measured under saturating light in normal samples. Still, in anticipation, our answer to the initial question: *Does the fluorescence induction in the presence of DCMU exhibit a thermal phase?* is "No" since, in our opinion, as we will discuss below, the theories supporting this idea (i.e., of Joliot and Joliot 1979; Vredenberg 2000, 2004; Schansker et al. 2011) do not have sufficient experimental support.

The thermal phase of the fast fluorescence induction: the J–I–P rise

In normal untreated samples, the maximum fluorescence yield F_M can be reached only after all the electron transport chain is reduced (Munday and Govindjee 1969a, b). This

would include the PQ-pool. Thus, it is reasonable to assume that the PQ-pool plays a role in the generation of the thermal phase. Several authors (Kramer et al. 1995; Prasil et al. 1996; Samson and Bruce 1996; Vasilev and Bruce 1998; Koblizek et al. 2001) have proposed that quenching at the J level (see Delosme 1967 for the original suggestion) is due to antenna quenching by oxidized plastoquinone, which was later shown to act as quencher in isolated chloroplasts (Vernotte et al. 1979; Haldimann and Tsimilli-Michael 2005). However, on the basis of the experiments of Tóth et al. (2005) (where it was shown that the redox state of PQ-pool does not influence the maximum fluorescence yield in intact leaves treated with DCMU), we could dismiss the idea that the thermal phase is caused by the reduction of oxidized plastoquinone from the PQ-pool.

Further, it is still possible that the J–I–P rise is influenced by oxidized, or partially reduced, PQ molecules occupying the Q_B-pocket (i.e., the so-called Q_B-quenching, or Q_B-occupancy theory; Schreiber 1986, 2002; Schreiber and Neubauer 1987; Schreiber et al. 1989; Vasilev and Bruce 1998; Kolber et al. 1998; see also Samson et al. 1999 and references therein). Since the majority of these alternative theories have already been fully discussed by Samson et al. (1999), we will present below only the salient points of an original hypothesis of Schreiber (2002), where Q_B is suggested to act as a quencher of fluorescence, and the process also involves the inactive branch of PSII RC. Finally, we will end our account on alternative theories with the presentation of those proposed by Vredenberg (2000, 2011) and by Schansker et al. (2011).

A Q_B-quenching hypothesis: does the inactive branch of PSII RC influence the Chl a fluorescence yield? Views of U. Schreiber

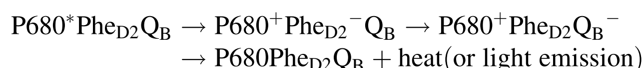
In DCMU-treated samples, when a PQ molecule cannot occupy the Q_B-pocket, the fluorescence yield reaches F_M with a rise time close to that of the O–J phase. On the other hand, in a dark-adapted untreated sample, the J level is lower than F_M, even if, in agreement with many alternative theories, starting with Delosme (1967), all PSII centers are closed at the end of the photochemical O–J phase. Since during the photochemical O–J phase, the Q_B pocket is occupied by a PQ molecule, it was suggested that the fluorescence at the J level is quenched by Q_B (Schreiber 1986; Schreiber and Neubauer 1987; Schreiber et al. 1989; Vasilev and Bruce 1998; Kolber et al. 1998; Samson et al. 1999; Schreiber 2002). The thermal J–I–P phase would then be due to a de-quenching process induced by a gradual PQ-pool reduction. Indeed, in the TEG model Q_B^- is considered tightly bound to the Q_B site, and both PQ and PQH_2 are assumed to have the same low affinity for the Q_B site (Velthuys and Amesz 1974; Crofts and Wraight 1983). As the PQ-pool becomes more and more reduced during the thermal phase, *the Q_B site will be empty for longer and longer time,* or, we can say that it will be visited more by PQH_2 molecules than PQ. It is also possible that PQH_2 cannot enter the Q_B site. PSII crystal structure shows the existence of two channels leading to the Q_B site: PQ may enter through one of them, and PQH_2 may leave through the other (see e.g., Guskov et al. 2009). There are also other factors that delay the exchange between PQH_2 and PQ, prolonging even more the period of time when the Q_B-site is not occupied by PQ (Robinson and Crofts 1984). We note that this theory, called Q_B-quenching theory (or also Q_B-occupancy theory, because the quenching takes place only when Q_B-site is occupied by a PQ molecule) is not contradicted by the results of Tóth et al. (2005), since in their experiments the Q_B-site was occupied by DCMU instead of Q_B.

In the majority of these theories, the mechanism responsible for Chl fluorescence quenching by Q_B is considered to be an antenna quenching (see Samson et al. 1999). A different explanation is, however, given in the theory of Schreiber (2002), which is presented below.

Schreiber (2002) considers that the low fluorescence yield after a saturating STF, F_m^{STF} or at the J level, can be explained if processes taking place both at the donor and acceptor sides of PSII are taken into account. It is known that treatments that slow-down the PSII donor-side activity, e.g., either UV irradiation (Yamashita and Butler 1968b), heat (Yamashita and Butler 1968b; Guissé et al. 1995), low pH in the lumen space (Krieger and Weis 1993; Johnson et al. 1995), Tris washing (Yamashita and Butler 1968a, 1969), or hydroxylamine (NH_2OH) (Bennoun and Joliot 1969; Bouges 1971), produce additional quenching of F_m^{STF} (and at the J level).

It seems likely that this donor-side quenching is caused by $P680^+$, since its reduction by Y_Z is slowed down after these treatments, as well as by the reopening of RCs by rapid charge recombination of $[P680^+Q_A^-]$ (Lavergne and Rappaport 1998). Therefore, donor-side dependent quenching requires long-lived $P680^+$, which is possible only if its reduction by Y_Z is slow. Under normal conditions, the reduction of $P680^+$ by Y_Z occurs very rapidly (in the sub-μs time range; Rappaport et al. 1994). However, this reduction rate is slowed down when a "double hit" occurs during the STF experiment, since the reduction of $P680^+$ after the second hit would be limited by the relatively slow re-reduction of Y_Z^+ by the OEC. A double hit, in a sufficiently long STF, will require, however, two acceptors per RCs (e.g., as those suggested by Joliot and Joliot 1973, 1977, 1979). Yet, with the current detailed knowledge of the PSII structure (Umena et al. 2011), there appears to be no room for an additional acceptor. Schreiber (2002) suggests that Q_B and Q_B^- can play this role of

second acceptor(s). They would be able to participate in charge stabilization, but only in a transient way, via a charge separation involving the 'inactive branch' of PSII RC (i.e., the D2 branch; see Fig. 1, and a review on PSII by Govindjee et al. 2010). However, this unusual primary charge separation between P680* and Phe_{D2} would occur only when Q_A is reduced (therefore leading to double hits). Schreiber (2002) assumed that the following reactions take place at the "inactive" branch of PSII (see Fig. 9):



$$P680^{*}Phe_{D2}Q_B \rightarrow P680^{+}Phe_{D2}^{-}Q_B \rightarrow P680^{+}Phe_{D2}Q_B^{-} \rightarrow P680Phe_{D2}Q_B + \text{heat(or light emission)}$$

Further, Schreiber (2002) has assumed that this charge separation, mentioned above, is followed by a rapid charge recombination driven by a strong local electric field, with two positive and two negative charges at the internal and external side of each RC, respectively. This recombination reaction, responsible for energy dissipation as heat or DLE, is assumed to be similar to that proposed by Lavergne and Rappaport (1998) for the inefficient Q_A reduction in the presence of DCMU and hydroxylamine. The period-2 oscillation of the fluorescence, due to charge accumulation associated with the two electron gate (TEG) mechanism (Velthuys and Amesz 1974), would not be disturbed, because a stable reduction of Q_B will take place only with a very low yield.

Schreiber (2002) considers this hypothetical "double hit process", involving a charge separation at the inactive branch of PSII RC and the reduction of Q_B (or Q_B^-) by Phe_{D2}^-, to be responsible for the well-known double hit discussed by Kok et al. (1970) (also see Shinkarev and Govindjee 1993), as well as for the quenching of the fluorescence yield after a STF. Since Q_B is suggested to be directly involved in this quenching reaction, the suppression of fluorescence quenching by PQ-reduction and by DCMU can be explained. Hydroxylamine would disconnect both the fast donor Y_Z, and the slow donor Y_D, and will act as a relatively slow donor to $P680^+$, thus causing a donor-side dependent quenching (Bouges 1971; Tóth et al. 2007a). The Q_B-quenching mechanism related to the inactive branch of PSII RC is, however, distinct from the donor-side dependent quenching, with the latter acting on top of the former, when the PSII donor-side is slowed down. Nevertheless, in order to explain the additional donor-side dependent quenching, we must assume that the Q_B-quenching is less efficient than Q_A and $P680^+$-quenching.

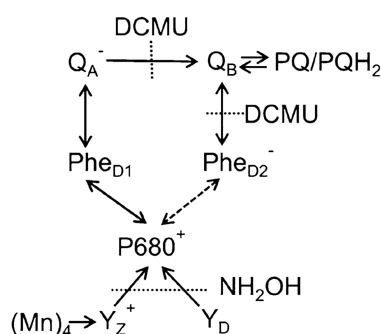


Fig. 9 A reaction scheme illustrating Q_B-quenching hypothesis proposed by Schreiber (2002); see details in the text. Q_A and Q_B are the primary and secondary plastoquinone electron acceptors; PQ/PQH_2 represents the PQ-pool; Phe_{D1} and Phe_{D2} are the Pheophytin molecules located on the D1-branch (active) and the D2-branch (inactive) of PSII core; DCMU is 3-(3′,4′-dichlorophenyl)-1,1′-dimethylurea; NH_2OH is hydroxylamine; P680 is the primary electron donor of PSII; Y_Z (i.e., D1-Tyr 161) and Y_D (i.e., D2-Tyr 160) are secondary electron donors; $(Mn)_4$ represents the Oxygen Evolving Complex. Modified from Schreiber (2002)

The above-described hypothetical process related to the inactive branch of PSII RC proposed by Schreiber (2002) is, however, highly speculative, and does not explain, for example, the low value of F_m^{STF} for short STFs, when double hits are improbable. Moreover, it seems improbable in our opinion that this kind of quenching can be responsible for a very large (40–50 %) part of the variable Chl *a* fluorescence.

Is Y_Z^+ a quencher? Can Phe^- accumulate under normal conditions? Which one is the quencher of Chl a fluorescence: Phe^- or Phe? Views of W. Vredenberg

Vredenberg and his coworkers (see Vredenberg 2000, 2004, 2008a, 2011; Vredenberg and Bulychev 2002, 2003; Vredenberg et al. 2006, 2012) have proposed an original, but controversial, theory, called the 'Three State Trapping Model' (TSTM) (see below), for the origin of the fluorescence rise during the fast FI, which is partially based on some of the ideas of Delosme (1967) and of Joliot and Joliot (1977, 1979, 1981a). Moreover, Vredenberg and coworkers have also presented a mathematical model, called 'Fluorescence Induction Algorithm' (FIA), in which an extended version of TSTM has been applied. They have simulated the O–J–I–P transient with FIA (Vredenberg 2008a, 2011; Vredenberg and Prášil 2009) using the function: $F^{FIA}(t) = F^{PP}(t) + F^{PE}(t) + F^{CET}(t)$, where $F^{PP}(t)$ is the fluorescence rise due to the release of primary photochemical quenching during the O–J phase, $F^{PE}(t)$ is the fluorescence rise due to the release of photoelectrochemical quenching during the J–I phase, and $F^{CET}(t)$ is the fluorescence rise during the I–P phase, associated with the fluorescence enhancement due to the CET-PSI (see details below).

The so-called TSTM advocated by Vredenberg (2000, 2004, 2008b) has the following assumptions and concepts:

(1) The closure of PSII RC requires not one, but two successive trapping events, leading to the reduction of

both the primary and secondary acceptors of PSII, Phe, and Q_A. The generation of a completely closed RC (P680Phe$^-$$Q_A$$^-$) was considered to have a quantum yield of at least 25 %. Therefore, in this model a PSII center can be in three states: open (with oxidized Q_A), semi-closed (with reduced Q_A), and fully closed (with reduced Phe and Q_A). (This definition of closed RCs had been earlier proposed for bacteria (Vredenberg and Duysens 1963).)

(2) The fluorescence yield of PSII antenna is under photoelectrochemical control that is exerted by the electric field in the vicinity of, and sensed by, the RC (see Vredenberg 2000, 2004). According to this concept, the positive charges on Y_Z and P680, and the negative charges on Phe and Q_A, participating to produce the local electric field, are cumulative. Considering "S" to be the algebraic sum of the electrical charges on these four components, there are three possibilities (Vredenberg et al. 2002): (a) $S = 0$, in which case it is assumed that the variable fluorescence $F_V = 0$, and the total fluorescence $F_{max} = F_0$; (b) $S = -1$, when it is assumed that $F_V = 2F_0$, and $F_{max} \sim 3F_0$; and (c) $S = -2$, when it is assumed that $F_V = 4F_0$, and $F_{max} \sim 5F_0$. For example, PSII states in the category $S = 0$, with $F_{max} = F_0$ are: Y_ZP680$^+$Phe$^-$$Q_A$, Y_ZP680$^+$PheQ_A^-, and Y_Z^+P680PheQ_A^-. Here the first two PSII states are usually considered non-fluorescent, due to the quenching effect of P680$^+$. For the last PSII state (Y_Z^+P680PheQ_A^-), however, Y_Z^+ appears to act as a quencher, as proposed earlier by Zankel (1973). Vredenberg (2004) considers that this hypothesis is consistent with what was experimentally observed by the fluorescence rise induced by a short (ns) saturating STF, when the fluorescence quenching continues in the microsecond domain, even after the main part of P680$^+$ quenching had been released (Mauzerall 1972). This quenching is usually attributed to ^{3}Car (see Steffen et al. 2001), but Vredenberg et al. (2002) have suggested that Y_Z^+ also contributes to quenching of fluorescence. PSII states when $S = -1$, with $F_{max} \sim 3F_0$ are: Y_ZP680PheQ_A^-; Y_ZP680$^+$Phe$^-$$Q_A$$^-$; and Y_Z^+P680Phe$^-$$Q_A$$^-$. We point out here that, in the last two PSII states, P680$^+$ and Y_Z^+ do not completely quench fluorescence. The only PSII state in the category $S = -2$, with $F_{max} \sim 5F_0$, is Y_ZP680Phe$^-$$Q_A$$^-$. Of course, every PSII state described above can contain OEC and Q_B in different redox states.

Vredenberg and coworkers have explained different phases of the O–J–I–P transient as follows.

The O–J phase (the photochemical phase): Vredenberg and coworkers arrived at the conclusion from their experiments that, in dark-adapted samples, there is a fraction of 25 % (or higher) of Q_B-nonreducing PSIIs (i.e., PSII centers that are unable to reduce Q_B; see Govindjee 1990); these Q_B-nonreducing centers are assumed to have OEC in the S_0 state (as S_0:S_1 ratio of OEC is known to be $\sim$25:$\sim$75 in dark-adapted samples; Kok et al. 1970). In his simplified reaction scheme for the photochemical phase, Vredenberg (2004) did not explicitly include the S states of OEC, Q_B and the PQ-pool. We note that Vredenberg and coworkers support the presence of a high initial proportion of Q_B-nonreducing centers, based on results obtained in simulation of the maximum fluorescence yield measured upon excitation with twin saturating flashes (labeled TTF; see Vredenberg et al. 2007). Vredenberg et al. (2007) assume that, under saturating continuous light, PSIIs are photoreduced to the 'semi-open' state (i.e., centers in [PheQ_A^-] state) at the end of the O–J phase of the fast FI in a proportion that is dependent upon $k_L/(k_L + k_{AB})$, where k_L is the apparent rate constant of Q_A reduction and k_{AB} is the rate constant of Q_A reoxidation by Q_B in 'semi-closed' centers (see Eq. 3).

We note that, according to Eq. 3, all active PSII centers can become semi-closed at the J level only if $k_L/(k_L + k_{AB}) = 1$ (i.e., for very high values of k_L, or when $k_{AB} = 0$). Moreover, at the same time, some of the initial Q_B-nonreducing RCs become fully closed (i.e., centers in [Phe$^-$$Q_A$$^-$] state) due to a double hit process, as assumed in TSTM (see above). The mathematical function used in FIA for the simulation of the O–J phase, derived from a simplified scheme, and based on the reactions presented in Fig. 10 is:

$$F^{PP}(t) = 1 + nF_V \cdot q^{dsq}(t) \cdot \left[(1-\beta)\cdot\frac{k_L}{k_L + k_{AB}} + \beta\cdot\left(1 + \left(1 - e^{-\Phi \cdot k_L \cdot t}\right)\cdot e^{-k_{2AB}\cdot t}\right)\right], \quad (3)$$

where $nF_V = (F_m{}^{STF} - F_0)/F_0$ is the normalized variable fluorescence; $q^{dsq}(t) = 1 - e^{-k_L \cdot t}$; β is the fraction of Q_B-nonreducing centers; Φ is an efficiency factor for energy trapping in Q_B-nonreducing centers with reduced Q_A; and k_L, k_{AB}, and k_{2AB} are the rate constants of photoreduction of Q_A, and of re-oxidation of $Q_A$$^-$ by Q_B in 'semi-closed' and 'fully closed' centers, respectively. The function $F^{PP}(t)$ was also used for the simulation of the fluorescence transient after a light pulse, or after treatment with DCMU.

One interesting result obtained with TSTM is that the fluorescence transient in the presence of DCMU can be simulated without assuming the existence of α- and β-centers, as had been proposed by Melis and Homann (1975) (see also Govindjee 1990 for a review on PSII

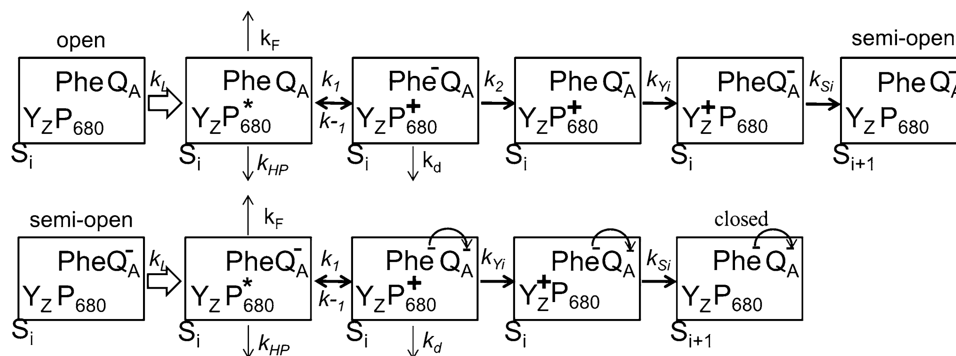


Fig. 10 *Top* Single-hit driven transfer of a dark-adapted 'open' photosystem II reaction center (PSII RC) with the Oxygen Evolving Complex (OEC) in the S_i state ($S_i[Y_ZP_{680}PheQ_A]$) into its first quasi-stationary 'semi-closed' form ($S_{i+1}[Y_ZP_{680}PheQ_A^-]$) in chloroplasts. *Bottom* Single-hit driven transfer of a 'semi-open' PSII RC with OEC in S_i state ($S_i[Y_ZP_{680}PheQ_A^-]$) into its quasi-stationary double reduced 'fully closed' form ($S_{i+1}[Y_ZP_{680}(PheQ_A)^{2-}]$). P_{680} is the first electron donor of PSII; Y_Z is the second electron donor of PSII; Phe is pheophytin; Q_A is the primary quinone electron acceptor; k_L is the rate constant of light excitaton; k_F is the rate constant of the radiative energy dissipation at the antenna level (fluorescence emission, delayed light emission); k_{HD} is the rate constant of nonradiative energy dissipation at antenna level; k_1 is the rate constant related to the intrinsic rate constant of primary charge separation; k_{-1} is the rate constant of radiative charge recombination that leads to re-excitation of antenna and ns Delayed Light Emission, DLE; k_d is the rate constant of the decay of the radical pair through nonradiative recombination to the ground state; k_2 is the rate constant of the decay of radical pair by electron transfer to Q_A; k_{Yi} is the rate constant of P_{680}^+ reduction by Y_Z, when OEC is in the S_i state; k_{Si} is the rate constant of Y_Z^+ reduction by OEC in the S_i state. (*Note*: The rate constants k_1, k_{-1}, and k_d have different values for the open than for the 'semi-closed' centers.) Modified from Vredenberg (2004)

heterogeneity). The area above the FI curve, which is usually used as a linear measure of the number of turnovers that are required for RC closure (Malkin 1966; Malkin and Kok 1966; Murata et al. 1966), is also considered invalid in Vredenberg's TSTM. Further, according to TSTM, the sigmoidicity of the fluorescence transient, usually associated with the connectivity between PSII units (Joliot and Joliot 1964), can be also caused by Y_Z^+ quenching. Indeed, the simulations presented by Vredenberg (2004) have shown that, when the apparent light excitation rate k_L has a value close to the rate constant of Y_Z^+ reduction by OEC in S_1 state (i.e., $\sim$10,000 s^{-1}), the FI curve becomes sigmoidal.

The J–I phase (a thermal phase): In Vredenberg' theory, the fluorescence rise during 2–30 ms time of the thermal phase is due to an accumulation of semi-closed Q_B-nonreducing RCs, which are considered to become fully closed (i.e., converted to a double reduced redox state $[Phe^-Q_A^-]$) with an efficiency as high as 50 %. This conclusion was based on experimental data obtained using trains of STFs in chloroplasts (Vredenberg et al. 2006, 2007), showing a gradual increase of F_M, similar to the J–I phase. This accumulation of semi-closed Q_B-nonreducing RCs was related to photoelectrochemical membrane responses to the pH gradient (ΔpH), generated across the thylakoid membrane during electron transport. The electrochemical response to the alkaline pH near stroma side of the membrane is assumed, in this theory, to modify the equilibrium constant of the reaction $Q_A^-Q_B \leftrightarrow Q_AQ_B^-$, favoring the back reaction, and therefore, leading to an accumulation of $Q_A^-Q_B$.

Further, Vredenberg and coworkers consider that these Q_B-nonreducing RCs are not irreversibly inactive, since they seem to be photo-converted into Q_B-reducing centers in a double hit photo-process (Vredenberg et al. 2006, 2007). However, these hypotheses, as well as the assumption of a high fraction of Q_B-nonreducing RCs in dark treated samples (≥25 %; see above for the explanation of the O–J phase), are in disagreement with experimental data showing that the fraction of Q_B-nonreducing centers in dark-adapted samples is considerably lower than 25 % (see Tomek et al. 2003; Schansker and Strasser 2005).

We note that in the mathematical model of the O–J–I–P transient by Vredenberg and coworkers, the oxido-reduction reactions of the PQ-pool are not included explicitly; they are considered only indirectly, as contributing to the transmembrane ΔpH building during the J–I rise. Since Vredenberg et al. (2006, 2007) have used isolated chloroplasts in their measurements, PQ-pool reduction can also influence the reported experimental data through its de-quenching (enhancement) effect (Vernotte et al. 1979; Haldimann and Tsimilli-Michael 2005). Therefore, our opinion is that the increase of F_M during the train of STFs observed by Vredenberg et al. (2006, 2007) is mostly due to the de-quenching effect induced by the reduction of the PQ-pool, rather than to the photo-conversion of Q_B-non-reducing centers in a double hit process. Moreover, the explanation suggested by Rappaport and coworkers (Boussac et al. 2011; Lavergne and Rappaport 1998) for the experimental data presented by Joliot and Joliot (1979,

1981a, b) (i.e., the re-oxidation of Q_A^- by oxidized NHI, fluorescence quenching by $P680^+$, and rapid charge recombination of $P680^+$ with Q_A^-) should also play an important role in the observed increase of F_M during the train of STFs reported by Vredemberg and coworkers.

The variable fluorescence during the J–I phase was fitted, in the first version of FIA (see Vredenberg 2008a), by the function:

$$F^{PE}(t) = nF_V \cdot \left[1 - e^{-k_{qbf} \cdot t} \cdot \sum_{m=0}^{N} \frac{(k_{qbf} \cdot t)^m}{m!}\right] \cdot (1 - \beta) \quad (4)$$

where k_{qbf} represents the rate constant attributed to pH change on the acceptor side of PSII, and N is an integer with $0 \le N \le 2$ that accommodates the delay and steepness of the J–(D)–I phase. In a later version of FIA (Vredenberg 2011), $F^{PE}(t)$ was calculated, for the particular case of $N = 0$, as:

$$F^{PE}(t) = 1 + nF_V \cdot \left\{[1 - f^{PPsc}(t)] \cdot (1 - e^{k_{qbf} \cdot t}) \cdot \frac{k_{qbf}}{k_{qbf} + k_{Hthyl}} + 1\right\} \cdot \left(1 - e^{-k_{qbf} \cdot t}\right) \cdot \frac{k_{qbf}}{k_{qbf} + k_{Hthyl}} \quad (5)$$

where f^{PPsc} is the fraction of semi-closed RCs (i.e., containing Phe and Q_A^-), and k_{Hthyl}, the actual passive transthylakoid proton leak (conductance) (see details in Vredenberg 2011).

The I–P phase (also a thermal phase): The fluorescence rise during the IP-phase was described by the following fitting function (Vredenberg 2011):

$$F^{CET}(t) = 1 + IP \cdot \left[1 - e^{-k_{IP} \cdot t} \cdot \sum_{m=0}^{N_{IP}} \frac{(k_{IP} \cdot t)^m}{m!}\right] \cdot \frac{k_{IP}}{k_{IP} + k_{Hthyl}} \quad (6)$$

where IP is the amplitude of the I–P phase, k_{IP} the rate constant of fluorescence rise in the IP-phase of FI, and N_{IP} an integer ($5 \le N_{IP} \le 12$) that accommodates the delay and steepness of the I–P phase. Vredenberg (2008a) believed initially that this function *hints* to a mechanism in which a signal is propagated via a number (NN) of approximately identical transfer steps from a distantly located generation site to a responsive target, which is probably connected with, and a reflection of an electrical interaction between PSI and PSII. The I–P phase was later suggested to be a response to an electrical field that is generated by the proton pump powered by CET-PSI and 'sensed' by the RCs of PSII, with k_{IP} and N_{IP} assumed to be related to properties of this proton pump (Vredenberg and Prášil 2009; Vredenberg 2011).

FIA was shown to successfully simulate different experimental O–J–I–P curves (Vredenberg and Prášil 2009; van Rensen and Vredenberg 2011). However, this does not prove the model proposed by Vredenberg and coworkers, since the built-in functions in FIA were especially designed to provide adjustments for a good fit (i.e., the terms $\sum_{m=0}^{N} \frac{(k_{qbf} \cdot t)^m}{m!}$ and $\sum_{m=0}^{N_{IP}} \frac{(k_{IP} \cdot t)^m}{m!}$ used in F^{PP} and F^{PE}, respectively, which *accommodate* the delay and steepness of the J–I and I–P phases; see details in Vredenberg 2008a), some of the constants were not constrained (i.e., k_{qbf}, k_{Hthyl}), and one is empirical (i.e., IP used in F^{CET}).

The model proposed by Vredenberg is highly original, and at the same time very complex. It has been received with skepticism because of several controversial points in its main hypothesis. They are:

(1) The assumption of a high quantum yield (i.e., even up to 50 %) for the photo-generation of the doubly reduced PSII RCs.

- This hypothesis is contradicted both theoretically and experimentally, by studies that show and/or predict a negligible (≤0.5 %; Klimov et al. 1977, 1980) or low (≤15 %; Schatz et al. 1988) quantum yield of this process.
- Further, the reduction of $P680^+$ after a second hit is limited by the relatively slow re-reduction of Y_Z^+ by the OEC, and therefore, the recombination between $P680^+$ and Phe^- is highly probable.
- In addition, as pointed out by Schreiber (2004), the accumulation of [$P680Phe^-Q_A^-$] is incompatible with the fact that maximum fluorescence yield is stable for hours in the dark, even in the presence O_2, when PSII is blocked by a combination of DCMU and hydroxylamine (Bennoun 1970).

(2) The assumption of quenching/de-quenching effect of oxidized/reduced Phe.

- Vredenberg's hypothesis contradicts the results that show that Phe in the reduced state, not in the oxidized one, is a quencher (Klimov et al. 1977; Shuvalov et al. 1980). Moreover, the reduced Phe photo-accumulation was shown to take place only under irradiation at 200–220 K (Klimov et al. 1980; Breton 1982), or under anaerobic conditions, when strong fluorescence quenching has been observed (Klimov et al. 1985, 1986; Heber et al. 1985); this quenching disappears with the addition of oxygen, indicating that the efficiency of the generation of reduced Phe is very low under normal conditions (see below).

- Further, the reduction of Phe causes absorbance changes around 820 nm, which can be measured in parallel with light-driven development of fluorescence quenching when O_2 is absent. However, in the presence of O_2, a corresponding absorbance change is not observed in parallel with light-driven fluorescence rise to F_M (Schreiber 2004).
- In order to counter this criticism, Vredenberg has proposed that the negative charge of Phe is displaced partially onto Q_A^-. However, there is no experimental data supporting this suggestion.

(3) The definition of photochemical activity at PSII level assumed in the TSTM, and the presence of a significant non-photochemical fluorescence enhancement active at the F_M level (see F^{CET}), implies a decrease in the value of the maximum quantum yield of primary PSII photochemistry, Φ_{PSII}, as measured using fluorescence data (for a different point of view on Φ_{PSII} see Vredenberg et al. 2012).

The model proposed by Vredenberg and coworkers, in our opinion, remains more or less speculative, as in addition to the above highly disputable points, other points of its hypotheses have not received convincing experimental proofs, in the studies of Vredenberg and coworkers, or of others.

We list below some of the ideas of Vredenbeg and coworkers that still need experimental confirmation:

(1) The specific photoelectrochemical control of the fluorescence yield, exerted by the local electric field generated by several components of the electron transport chain, is located in close vicinity of the PSII center (i.e., Y_Z, P680, Phe, and Q_A).
(2) Y_Z^+ acts as a quencher of Chl *a* fluorescence.
(3) The existence of an unusually high fraction of Q_B-nonreducing centers in dark-adapted samples, and its increase during the J–I phase, due to the building of transmembrane ΔpH; also, the double photo-reduction of these Q_B-nonreducing centers with an efficiency >50 % during the J–I phase.
(4) The origin of the I–P phase, as being due to a fluorescence enhancement induced by the electric field generated during CET-PSI activity (see the above explanation for the I–P phase); moreover, we wonder why there is no fluorescence enhancement assumed by Vredenberg and coworkers before the I–P phase, as the membrane potential ΔΨ is known to increase also during the J–I rise (see the model proposed by Pospíšil and Dau 2002).

Can the J–I–P phase be the result of a conformational change? Views of I. Moya, of G. Schansker, and of F. Rappaport

Moise and Moya (2004a, b) had accepted the idea of Delosme (1967) that the photochemical and thermal phases are separated under continuous saturating light, and proposed that a conformational change takes place during the I–P phase. Moise and Moya measured Chl *a* fluorescence lifetime (τ) and yield (*Φ*), by a phase-modulation method, during FI in leaves under actinic light of low intensity (100 µmol photons m^{-2} s^{-1}); they identified three typical phases of τ–*Φ* relationship: (a) linear during the O–I rise; (b) convex curvature during the subsequent I–P thermal rise; and (c) linear during the P–S slow fluorescence decrease. Moreover, they showed that the time dependence of the far-red fluorescence band (F735; mostly from PSI) versus the red band (F685; mostly from PSII) also deviates from a straight line during the I–P rise of the OIDPS transient. The peak at 685 nm showed a transitory and variable blue shift during the I–P rise, whereas the position of the far-red peak at 735 nm remained unchanged (Moise and Moya 2004a). This effect had been already reported earlier (e.g., Malkin et al. 1980), and two different interpretations had been given: rapid energy distribution changes between PSII and PSI (Schreiber and Vidaver 1976), and an optical effect of light diffusion and re-absorption of fluorescence (Malkin et al. 1981; Peterson et al. 2001). However, Moise and Moya (2004a, b) dismissed these explanations based on spectrally resolved analysis. They advocated a *variable and transitory* non-photochemical quenching (NPQ) to be taking place during the I–P phase of the fluorescence transient, due to a transitory change in energy dissipation related to a conformational change at the level of auxiliary antenna CP47 of the PSII RC core complex; they were able to simulate this hypothetical mechanism using a modified RRP model.

We note, however, that Bradbury and Baker (1983), based on measurements of fast FI curves on normal and DCMU-treated samples at 685 and 740 nm, have also proposed the presence of a variable NPQ accompanying the photochemical quenching, but during the O–I rise. Moise and Moya (2004a) suggested that their hypothetical conformational change was not removed by atrazine and DCMU addition, but it disappeared at low temperatures, since the τ–*Φ* relationship during the IP phase became linear at −50 °C. This last observation is consistent with the results reported by Butler and Strasser (1977a), which showed that, irrespective of the chloroplast concentration, at low temperatures (−196 °C), there is a linear relation between the fluorescence measured at 694 and 730 nm during the FI. At the same time, the "spillover" of

excitation energy from PSII to PSI was shown to take place even at low temperatures (Satoh et al. 1976), and therefore this process cannot be considered to be the origin of the τ–Φ and Φ_{685}–Φ_{735} curvature, as suggested by Schreiber and Vidaver (1976). In addition, Moise and Moya (2004a) observed, based on the above experimental data, that the quenching by oxidized PQ-pool is essentially dynamic, as it affects both the fluorescence yield and lifetimes (Eftink 1991). A static quenching by the oxidized PQ-pool, as proposed by Kurreck et al. (2000), would have left the fluorescence lifetime unchanged.

Schansker et al. (2011) have also suggested that a conformational change takes place during the thermal phase of the fast FI. They measured it in saturating light, and suggested that it accounts for ~30 % of the variable chlorophyll fluorescence. They used dark relaxation kinetics of the fluorescence yield after a saturating multi-turnover light pulse, measured in the presence or the absence of DCMU, to study this hypothetical conformational change. The fluorescence decay at room temperature of DCMU-treated samples was fitted with two exponentials of $\tau = 72$ ms (~30 % amplitude), and $\tau = 740$ ms (~70 % amplitude). The slow phase was attributed to the recombination between Q_A^- and S_2 state of the OEC. Further, fluorescence decay from F_M, this time measured in untreated samples, showed two rapid relaxation phases during the first 100 ms decay (of ~30 % total amplitude), and two slower relaxation phases from 100 ms to 200 s (~70 %). Schansker et al. (2011) assigned the slower phases to the recombination of Q_A^- with S_2 and S_3 states of OEC, and to the forward electron transfer towards a slowly re-oxidizing PQ-pool in the dark in PSII centers, with the OEC in S_0 and S_1 states. However, they were not able to assign the fastest phases, with τ values of 6 ms (15 %) and 42 ms (13 %), as they were considered too rapid to be ascribed to either Q_A^- re-oxidation by a charge recombination within PSII, or the forward electron transport (due to the presence of a completely reduced PQ-pool at the end of the O–J–I–P(= M) transient).

Further, based on the quasi-similar relaxation times and amplitudes for the fast phases measured in the presence or the absence of DCMU, Schansker et al. (2011) assumed that a conformational change takes place during the end of the thermal phase, which is reversed in the first 80–100 ms of darkness after a saturating light pulse that is responsible for 30 % decline of the fluorescence yield. We note, however, that neither direct measurements, nor any precise description of what this conformational change is, are available to us. We ask: how a conformational can affect fluorescence? At least two possibilities can be considered: a conformation change can (a) alter the fluorescence yield emitted by Chl antenna (by modifying the amount of the absorbed light, or the fraction of the absorbed energy dissipated through non-radiative processes—see Fig. 8) (as assumed by Moise and Moya 2004a, b); or (b) may influence the kinetics of the photochemistry at PSII RC level (see also the discussion at the end of the section "Numerical simulation of the fluorescence transient induced by a saturating STF: is the rate of nonradiative recombination between $P680^+$ and Phe^- the key parameter for a lower F_m^{STF} value than F_M?").

Schansker et al. (2011) have shown that, in normal samples, the hypothetical process associated with the thermal phase could be regenerated within 2–3 ms rise time by a second saturating light pulse applied after 100 ms dark relaxation from F_M, and that the process is dependent on light intensity. They assumed that in normal chloroplasts the induction of the conformational change necessitates the reduction of all Q_A as a precondition, and that it relaxes rapidly in darkness *while Q_A is still completely reduced*. On the other hand, in samples treated with DCMU, the induction of the conformational change is assumed to occur gradually, in parallel with the reduction of Q_A, because Q_A^- cannot be re-oxidized by Q_B in this case. This would lead to an overlap between the fluorescence rise due to Q_A reduction, and the fluorescence rise due to the conformational change.

Further, Schansker et al. (2011) suggested that the well-known sigmoidicity of DCMU transients is not related to the energetic connectivity between PSIIs, as originally proposed by Joliot and Joliot (1964), but to the overlap between the hypothetical thermal phase and the photochemical phase of the fluorescence transient, negating at the same time the sigmoidicity of O–J–I–P curves measured in normal samples. They showed further that the sigmoidicity of DCMU transient disappeared at −10 °C, and suggested that this was due to the separation between the thermal and photochemical phase of the fast FI at low temperatures. We point out, however, that although Schansker et al. (2011) did not observe sigmoidicity of the FI curve in untreated samples, there are many reports in the literature in which such a sigmoidicity is in fact observed (e.g., Strasser and Stirbet 2001; Mehta et al. 2010, 2011). Moreover, the connectivity between PSII units is a phenomenon that has been shown to influence processes other than FI, for example, thermoluminescence (Tyystjärvi et al. 2009); and this must not be ignored.

In order to study the presumed separation between the hypothetical thermal phase and the photochemical phase, Schansker et al. (2011) measured the FI curves in DCMU-treated samples at temperatures ranging from +20 to −80 °C. They showed that, as the temperature was lowered, the rise time of the fluorescence to its maximum value became progressively longer. For temperatures until −10 °C, it was possible to fit the DCMU FI curves with two exponentials, the amplitude of the first phase

decreasing, while that of the second phase increasing with decreasing temperatures. Schansker et al. (2011) interpreted this observation to indicate a gradual separation between the photochemical and thermal phases. However, at temperatures below −20 °C, a third slower exponential component appeared. The rise time of the fastest phase was found to be nearly temperature independent and was thus, attributed to the photochemical phase. The other two phases were strongly temperature-dependent, and were assigned to either the thermal phase, or to the unstabilized charge separation occurring at low temperatures.

In our opinion the above interpretations of the complex changes of FI curves, in the presence of DCMU, and at low temperatures, do not necessarily prove the existence of a thermal phase, since they were accepted without consideration of other processes that may interfere with fluorescence emission, specifically at subfreezing temperatures, as, e.g., (a) existence of different types of PSII heterogeneity (see e.g., Govindjee 1990); (b) structure-related changes at low temperatures; (c) possible OEC activity impairment and activation of alternative electron transport pathways (as CET-PSII) (Brudvig et al. 1983, Schlodder 2008); and (d) possible DLE contribution to the variable fluorescence, which is known to be temperature-dependent (Tyystjärvi and Vass 2004).

Further, the other argument given by Schansker et al. (2011) in favor of a conformational change, which enhances the fluorescence yield during the thermal phase, is based mainly on the fact that they were not able to assign the two fast phases of the dark fluorescence relaxation from F_M to any known reoxidation reaction of Q_A^-, since they were just too fast. However, we will show below that these fast fluorescence relaxation phases can in fact be explained adequately by the reoxidation of Q_A^-.

The experimental data of F. Rappaport contradict the conclusion of Schansker et al. (2011) discussed above. We note the following:

(1) The recombination between S_2 and Q_A^- in DCMU-treated samples, the main reaction leading to dark fluorescence decay in this case, follows generally a heterogeneous decay (Bennoun 1970), not a simple first order time course (as assumed by Schansker et al. 2011).

(2) Rappaport et al. (2002, 2005) showed that the fluorescence decay from F_M is hyperbolic; they suggest that it is due to energetic connectivity between several PSII centers (Joliot and Joliot 1964). Consequently, the apparent half-times (τ) of the recombination kinetics of $S_2Q_A^-$ state calculated from these curves will have smaller values than the actual ones. This will explain the unusual low τ values of the fast phases of dark fluorescence dacay from F_M measured by Schansker et al. (2011).

(3) Cuni et al. (2004), using a weak flash exciting only ~16 % of PSII centers, have obtained ~3.7-fold longer apparent half-times for the recombination between S_2 and Q_A^- in the presence of DCMU, than when monitoring the fluorescence decay after a saturating flash. The difference was attributed to the elimination of the hyperbolic correlation between the fluorescence yield and $[Q_A^-]$ (see Eq. 1), due to the presence of a mostly open PSIIs population after the weak light flash.

(4) Further, using the weak light flash technique, Cuni et al. (2004) have shown that, for wild type (WT) *Chlamydomonas reindhardtii*, the recombination kinetics of $S_2Q_A^-$ state at room temperature is fitted quite well by a sum of *two* exponentials, with the fast phase accounting typically for ~35 % of the amplitude, with $\tau < 1$ s, and the slow phase about five times slower.

In view of the above information, we suggest that the initial ~30 % of the fluorescence decay in DCMU-treated pea leaves reported by Schansker et al. (2011) can be attributed to the fast phase of the recombination kinetics of $S_2Q_A^-$ state, and therefore, to Q_A^- re-oxidation.

We follow the same arguments for the interpretation of the fluorescence decay from F_M in normal samples, as those suggested above for DCMU-treated samples, because at the F_M level, PQ-pool is completely reduced, and consequently, the reoxidation of Q_A^- is also blocked. Hence, we believe that that the τ values of 6 ms (15 %) and 42 ms (13 %) reported by Schansker et al. (2011) for normal samples, can also be attributed to the charge recombination of PSIIs in $S_2Q_A^-$ state.

We conclude that the experimental data of Schansker et al. (2011) can be explained in a more credible way by Q_A^- re-oxidation via $S_2Q_A^-$ recombination reaction, than by a hypothetical conformational change. Thus, we cannot accept the conformational change hypothesis proposed by Schansker et al. (2011) at this time to be the main mechanism for the thermal J–I–P rise.

Further, the results obtained in the experiment with two successive saturating light pulses separated by 100 ms dark period, as used by Schansker et al. (2011) for the study of their hypothetical conformational change, can be interpreted differently if Q_A^- re-oxidation is held responsible for the initial rapid fluorescence decay from F_M. Indeed, in this case, the second light pulse is expected to gradually photoreduce the fraction of Q_A re-oxidized during the dark relaxation period, until F_M is reached again. Moreover, the dependence of fluorescence rise on light intensity of the

second pulse, as observed experimentally by Schansker et al. (2011), becomes obvious, if attributed to Q_A photoreduction. We note, however, that the above interpretation of these experimental data is compatible not only with the theory of Duysens and Sweers in its modified version, but also *with the alternative theories advocating PQ-pool quenching and Q_B-quenching* at the origin of the J–I–P rise, if we accept the idea that the PQ-pool is still completely reduced when the second light pulse is administered, as proposed by Schansker et al. (2005) and Tóth et al. (2007a, b).

Which theory is right, the theory of Duysens and Sweers in its modified version, or the alternative theories? Arguments pros and cons and a summary of all available results

In Table 1 we present a summary of the main interpretations of the O–J–I–P transient measured in saturating light, and discussed in this review, where we specify the fluorescence quenchers and enhancers that were suggested to influence the fluorescence yield, and the redox state assumed for Q_A and PQ-pool during the O–J, J–I, and I–P phases. The redox state of the acceptor side of PSI is also shown, but only for those models that have considered it explicitly. As we can see, the central difference between the theory of Duysens and Sweers in its modified version and the alternative theories is that Q_A reduction is completed at the end of the transient (the F_M level), or at an earlier time (see "Modifications to the theory of Duysens and Sweers (1963)" section). Schansker et al. (2011) correctly state that the complete reduction of Q_A is not attained at the J level for saturating light intensities used in the majority of commercial fluorometers, since in those measurements the fluorescence yield during the J–I rise does not show saturation (see Fig. 5), but they assume that it takes place during the J–I rise (closer to the J level at higher light intensities) based on results obtained from simultaneous measurement of fluorescence and μs DLE transients. We have discussed this issue earlier (see "The reduction of Q_A during the O–J phase: are all PSIIs closed at the J level?" section), and have shown that several other interpretations of μs DLE data are possible, which by contrast, confirm the idea of Duysens and Sweers, of a complete Q_A reduction only at the end of the transient (Itoh 1980; Goltsev et al. 2009).

In our view, there is no credible experimental evidence yet demonstrating that, under saturating light used in the majority of commercial fluorometers, Q_A is completely reduced before the maximum fluorescence yield is reached, and it seems that this issue is most often overlooked, and had been considered as a dogma in the majority of the alternative theories to the modified version of Duysens and Sweers Theory.

In many alternative theories, it is assumed, that besides Q_A, a second quencher exists: e.g., "R", Delosme 1967; or "Q_2", Joliot and Joliot 1979, 1981a; with "R" and "Q_2" unspecified. In addition to these hypothetical quenchers, the following specific quenchers have been suggested (see Table 1): (1) oxidized PQ-pool (Vernotte et al. 1979; Kramer et al.1995; Prasil et al. 1996; Samson and Bruce 1996; Pospíšil and Dau 2002); (2) Q_B itself (Schreiber et al. 1989; Samson and Bruce 1996; Vasilev and Bruce 1998; Kolber et al. 1998; Samson et al. 1999); (3) oxidized Phe (Vredenberg 2000); and (4) $P680^+$, which is supposed to accumulate due to an assumed photochemical activity of the inactive branch of PSII RC, leading to the photoreduction of Q_B (or Q_B^-) by a 'second hit' following the reduction of Q_A (Schreiber 2002). Moreover, non-photochemical fluorescence enhancement processes were also considered at the origin of the thermal phase, as: (1) radiative recombination of $P680^+$ with Phe^- (i.e., ns DLE; Schreiber and Krieger 1996); (2) influence of membrane potential ($\Delta\Psi$) during the J–I phase on the kinetics of the primary charge separation reaction (Pospíšil and Dau 2002); (3) influence of membrane potential ($\Delta\Psi$) during the I–P phase, as controlled by CET-PSI (Vredenberg 2011); and (4) unspecified conformational change, being responsible for $\sim$30 % of the variable fluorescence, F_V (Schansker et al. 2011).

The non-photochemical quenching by the oxidized PQ-pool was shown to influence the fluorescence yield only in samples in which the integrity of the photosynthetic apparatus was affected (e.g., isolated thylakoids) (Tóth et al. 2005), and therefore it may not be the cause of the thermal phase, acting only as a modifier in those special cases (see "Modulation of the fast FI by processes or components of the photosynthetic apparatus other than Q_A" section; see also Stirbet et al. 1998; Lazár 2009). (However, as mentioned above, further research is needed to reexamine this issue.)

In the models of Samson and Bruce (1996), and Schreiber (2002), the Q_B-site occupancy by an oxidized (or partly reduced) PQ is assumed to play a major role during the J–I–P thermal phase, since Q_A is considered to be completely reduced at the J level (Delosme 1967); the fluorescence would increase gradually, as the PQ-pool reduction proceeds, the maximum being attained only when the Q_B site is not occupied by an oxidized or by partially reduced PQ; this happens when PQ-pool is fully reduced.

On the other hand, in the model proposed by Schreiber and Krieger (1996), the J–I–P rise is due to a gradual increase of ns DLE induced by an unknown process

Table 1 Different interpretations of the chlorophyll *a* fluorescence transient, the O–J–I–P phase, measured in saturating light. In vertical column 1, we have listed the names of those who gave the original idea; in vertical column 2, and in 2nd and subsequent horizontal rows, we have listed authors who have further extended these ideas

	Fluorescence (FL) quencher(s)	Fluorescence (FL) enhancer(s)	Explanation of the O–J phase	Explanation of the J–I phase	Explanation of the I–P phase
Duysens and Sweers (1963)	Q_A (Duysens and Sweers 1963)	–	FL rises from F_0 to F_M due to gradual reduction of Q_A, until all the active PSIIs are closed at F_M		
	Q_A (Munday and Govindjee 1969a, b)	–	FL rises from F_0 to F_I due to (partial) Q_A reduction		FL rises until all the active PSIIs are closed at F_M; PSI acceptor side reduction modulates the I–P phase
Delosme (1967)	Q_A; and R (unidentified) (Delosme 1967)	–	FL rises due to Q_A reduction in all the active PSIIs	FL rises due to the disappearance of R, which is related to PQ-pool reduction	
	Q_A; and PQ in PQ-pool (Vernotte et al. 1979)	–	FL rises due to Q_A reduction in almost all the active PSIIs	FL rises due to de-quenching induced by PQ-pool reduction; total PQ-pool reduction occurs at F_M	
	Q_A and Q_B (Schreiber et al. 1989; Samson and Bruce 1996)	–	FL rises due to Q_A reduction in almost all the active PSIIs	FL rise is related to Q_B-quenching; Q_B-quenching is eliminated by total PQ-pool reduction at F_M	
	Q_A; and $P680^+$ (related to Q_B-site occupancy) (Schreiber 2002)	–	FL rises due to Q_A reduction in almost all the active PSIIs	FL rise is related to Q_B-site occupancy	FL rises until Q_B-quenching is eliminated at F_M, due to total PQ-pool reduction
Joliot and Joliot (1979, 1981a)	Q_1(= Q_A); and Q_2 (unidentified) (Joliot and Joliot 1979, 1981a)	–	FL rises due to Q_A reduction in almost all the active PSIIs	FL rises due to Q_2 reduction	
Schreiber and Krieger (1996)	Q_A; high rate of dissipative reactions at the RC level (Schreiber and Krieger 1996)	ns DLE	FL rises due to Q_A reduction in almost all the active PSIIs; FL quenching is due to nonradiative recombination of $P680^+$ with Phe^-	FL rises due to gradual increase in ns DLE induced by a decrease in nonradiative recombination of $P680^+$ with Phe^- (due to unknown processes, probably related to Q_B-occupancy)	
Vredenberg (2000), Bulychev and Vredenberg (2001)	Q_A; $P680^+$; $Y_Z{}^+$; and Phe (Vredenberg 2000, 2011)	Membrane potential ($\Delta\Psi$)	FL rises due to Q_A reduction (in a proportion depending on the light intensity and the rate of $Q_A{}^-$ reoxidation)	FL rises due to complete reduction of Q_A, and to the increase of the fraction of Q_B-nonreducing centers that have also reduced Phe	FL rises due to $\Delta\Psi$ related to CET-PSI activity
	Q_A; and PQ in PQ-pool (Pospíšil and Dau 2002)	Membrane potential ($\Delta\Psi$)	FL rises due to total Q_A reduction in all the active PSIIs	$\Delta\Psi$ stimulates the FL yield	FL rises due to de-quenching induced by PQ-pool reduction
Moise and Moya (2004a, b)	Q_A (Schansker et al. 2011)	Conformational change (unknown location or cause)	FL rises due to partial Q_A reduction	FL rises due to Q_A reduction in all the active PSIIs, and to a conformational change that is induced when PQ-pool and PSI acceptor side are also reduced	

(eventually correlated with Q_B-site occupancy), which regulates the rate constant of non-radiative charge recombination of the primary radical pair [$P680^+Phe^-$]. Q_B-quenching theories are not contradicted by the results presented by Tóth et al. (2005). However, in neither of these theories the correlation between the J–I–P rise and Q_B-site occupancy has a generally accepted experimental proof.

Vredenberg (2000, 2004) considers the oxidized Phe to be the major second quencher, and at the same time acknowledges the quenching effect of $P680^+$ and Y_Z^+ at the beginning of the fluorescence transient. Further, the membrane potential generated by CET-PSI is assumed to enhance the fluorescence yield during the I-P phase of the FI. However, the quantum yield for the photogeneration of doubly reduced PSIIs, i.e., $P680Phe^-Q_A^-$, is predicted and/or measured to be very low (Klimov et al. 1977, 1978). Moreover, most of the researchers do not consider the oxidized Phe to be a quencher, but assume that, like in special preparations under anaerobic and reducing conditions, its reduced form (Phe^-) is a quencher (Klimov et al. 1977; Shuvalov et al. 1980).

Finally, some of the alternative models analyzed in this review suggest a variation of the fluorescence yield during the thermal phase induced by a conformational change (Moise and Moya 2004a, b; Schansker et al. 2011). This conformational change is considered blocked at low temperatures by Moise and Moya (2004a, b), but not by Schansker et al. (2011). Moise and Moya (2004a, b) correlated the curvature of the τ–Φ (lifetime–quantum yield of fluorescence) relationship, measured during the IP phase, with a variable, transient conformational change that would take place in the pigment–protein complex of PSII core antenna. We note, however, that Moise and Moya based their theory on fluorescence measurements at low light intensity, when the FI (i.e., OIDPS) is not saturated; which is why we have not included this theory in Table 1. On the other hand, Schansker et al. (2011) presented results on fluorescence decay from F_M, which demonstrate, in their opinion, the impossibility of Q_A reoxidation during the first 100 ms of fluorescence relaxation in darkness; that would prove to them the involvement of a photoinduced conformational change during the J–I–P phase, responsible for 30 % of the variable fluorescence. However, we have pointed out in this review that this is not the case.

Our opinion, after we have critically analyzed these different models, is that the most credible interpretation of the O–J–I–P transient (and the thermal phase) is the modified version of Duysens and Sweers Theory, which considers that, upon illumination of a dark acclimated sample with saturating light, the Chl *a* fluorescence rises from its minimal level F_0 to the maximal level F_P (= F_M) in parallel with the de-quenching process induced by the progressive complete photoreduction of Q_A. This idea was extended by Munday and Govindjee (1969a, b); they demonstrated that even though the variable fluorescence originates only from PSII antenna, the fast induction curve is also influenced (i.e., modulated) by the activity at PSI level, specifically the J–I–P thermal phase.

The presence of several quenchers and modifiers that influence the fluorescence yield during the O–J–I–P transient is well established; thus, we have to accept a nonlinear relationship between F and [Q_A], and not a linear one, as was originally suggested by Duysens and Sweers (1963). However, results obtained through mathematical modeling of the fast FI (see e.g., Stirbet et al. 1998; Lazár 2003; Belyaeva et al. 2003; Zhu et al. 2005; Laisk et al. 2009a, b; Lazár 2009; Rubin and Riznichenko 2009; Lazár and Schansker 2009), have shown that these quenchers and processes generally do not disrupt in a drastic way the characteristic trend of the O–J–I–P curve as measured in dark-adapted samples. The main processes considered in the simulations of fluorescence transients by modeling include, e.g.: energy transfer and dissipation in PSII antenna and primary photoreactions (through RRP models); quenching by $P680^+$ and ^3Car; PSII connectivity; S states of the OEC; PQ-pool non-photochemical quenching; heterogeneity of the PQ-pool size or PSII centers; Cyt b_6/f; PSI; factors (such as ΔpH and local or transmembrane $\Delta\Psi$) that influence the rate constants of some redox reactions; photophosphorylation; alternative electron transfer paths (e.g., CET-PSI, CET-PSII, WWC); or even some other metabolic dark reaction (see "Mathematical simulation of the O–J–I–P transient: is it possible to describe both the photochemical and thermal phases based on the modified version of Duysens and Sweers Theory?" section). Results obtained using structure-based mathematical models have confirmed that PSI activity (via Cyt b_6/f) plays an essential role during the J–I–P rise (in agreement with the earlier conclusions of Munday and Govindjee 1969a, b). Moreover, with such a model it has been possible to simulate successfully not only the O–J–I–P transient but also the absorbance changes at 820 nm, as measured with control-, MV-, and DBMIB-treated samples, and with different intensities of excitation light (Lazár 2009).

Another argument in favor of the theory of Duysens and Sweers, and against some of the alternative theories (see below), is related to the maximum quantum yield of primary PSII photochemistry, Φ_{PSII}. Φ_{PSII} has been estimated from $F_V/F_M = (F_M - F_0)/F_M$ (Butler and Kitajima 1975; Govindjee 1995, Strasser et al. 2000, 2004) based on the assumption that the fluorescence rise to its maximum value F_M is only the result of Q_A reduction in all the active PSII centers. In most higher plants, the normal value of Φ_{PSII} obtained with this formula is in the range of 0.78–0.84, with a medium value of 0.83 for C3 plants (Björkman and Demmig 1987). These values were found to be in agreement with the maximum photochemical yield of photosynthesis estimated from the initial slope of the light saturation curve of O_2 evolution, Φ_{O_2} (Björkman and Demmig 1987; Long et al. 1993). Therefore, the formula $\Phi_{PSII} = F_V/F_M$ has an established validity, and it is used by a vast majority of researchers in the field today (for a different opinion, see Vredenberg et al. 2012). As this

formula was determined based on the assumption that the variable fluorescence F_V is due only to Q_A reduction in all the active PSII, it leads to the conclusion that, besides the photochemical quenching (i.e., the Q_A quenching), all various types of non-photochemical quenching (or enhancement) that affect the fluorescence yield during the O–J–I–P(= M) rise should be released when F_M is reached (see the modified version of the Duysens and Sweers Theory in "Modifications to the theory of Duysens and Sweers (1963)" section). However, according to Samson et al. (1999), the fluorescence data must be first corrected for the contribution of PSI fluorescence, since at wavelengths greater than 700 nm, typically used in commercial fluorometers, the constant PSI fluorescence contributes significantly to the total fluorescence (Genty et al. 1990; Lavergne and Trissl 1995; Pfündel 1998; Gilmore et al. 2000; Rappaport et al. 2007). Further, Samson et al. (1999) show that Φ_{PSII} calculated using the maximum fluorescence measured after saturating STFs, $F_m{}^{STF}$, instead of F_M, should give accurate Φ_{PSII} values after correction for PSI contribution.

In Table 2, we show Φ_{PSII} values for C3 plants, estimated before and after the correction of F_0 and F_{max} for constant PSI fluorescence contribution (i.e., 30 % of F_0 for C3 plants; Pfündel 1998). It can be seen that, even after correction, Φ_{PSII} determined on the basis of $F_m{}^{STF}$ is still underestimated, while that determined on the basis of F_M is only slightly increased (i.e., by 5 %).

In our opinion, the idea to use $F_m{}^{STF}$ *to estimate* Φ_{PSII}, instead of F_M, is a mistake, since Samson et al. (1999) had also assumed the presence of a non-photochemical quenching by the oxidized PQ-pool (or the presence of Q_B-quenching) at $F_m{}^{STF}$ level. On the other hand, the small increase (i.e., ~ 5 %) of Φ_{PSII} measured based on F_M, after PSI correction (see Table 2) implies that even if we accept that some non-photochemical process enhancing the fluorescence yield (e.g., a conformational change, or $\Delta\Psi$) can be still acting when the fluorescence reaches F_M, its contribution to the variable fluorescence F_V would be very small. Therefore, from this point of view, the alternative theories suggesting an increase of the fluorescence yield that is not correlated with the O_2 evolution by the OEC, as those proposed by, e.g.: Schreiber and Krieger (1996), Pospíšil and Dau (2002), Vredenberg (2011), and Schansker et al. (2011), would lack credibility, even after correction for PSI.

Finally, as the interpretation of O–J–I–P transient based on the modified version of Duysens and Sweers Theory is the mainstream idea in the field, many experimental or theoretical results, are in agreement with this idea. For example, we have shown earlier that:

(1) Data on maximum quantum yield of primary PSII photochemistry Φ_{PSII} support this picture (see above);
(2) Results obtained by Schansker et al. (2011), in experiments in which µs DLE induction curves and fast FI transients were measured simultaneously, are compatible with this theory (Itoh 1980; Goltsev et al. 2009); and
(3) Fluorescence decay data published by Rappaport et al. (2002, 2005) and Cuni et al. (2004) also support this theory.

Further, we can add to this list the theoretical analysis of the O–J–I–P transient, developed by Strasser and coworkers (Strasser and Strasser 1995; Strasser et al. 2000, 2004; Tsimilli-Michael and Strasser 2008), dubbed as 'the JIP-test'. This test is based on the original ideas of Duysens and Sweers (1963), and uses major inflection points of the fast FI curve to calculate a set of parameters characterizing the structure and photochemical activity of photosynthetic samples. One of the many parameters defined in the JIP-test is related to the thermal phase, as it correlates the reduction of PSI acceptors with the I step (Tsimilli-Michael and Strasser 2008). In spite of some limitations due to the use of a number of approximations (see a review by Stirbet and Govindjee 2011), the practical use of this model has clearly demonstrated that it can explain and predict well the performance of photosynthetic samples under several, if not all, conditions, especially when it is used in parallel with other measurement techniques besides FI (see e.g.,

Table 2 The maximum photochemical yield of PSII photochemistry, $\Phi_{PSII} = F_V/F_M$, calculated from a typical O–J–I–P fluorescence transient measured in pea leaf at room temperature, under illumination with 3,200 µmol photons m^{-2} s^{-1} (wavelength, 650 nm) (when $F_{max}/F_0 \approx 6$), or from fluorescence induction (FI) measured after single saturating flashes (STFs) with the pulse duration Δt in the microsecond (when $F_{max}/F_0 \approx 3$) or nanosecond (when $F_{max}/F_0 \approx 2$) range

FI experiment	F_{max}	F_0	F_{PSI} in C_3 plants	F_0 corrected	F_{max} corrected	$\Phi_{PSII} = F_V/F_M$	Φ_{PSII} corrected
O–J–I–P	3,000	500	$F_{PSI} = 30\ \%\ F_0 = 150$	350	2,850	0.83	0.88
STF ($\Delta t \approx$ µs)	1,500				1,350	0.67	0.74
STF ($\Delta t \approx$ ns)	1,000				850	0.50	0.59

The Φ_{PSII} values are estimated before, and after correction of F_0 and F_{max} for the constant PSI fluorescence contribution (F_{PSI}) (i.e., 30 % of F_0 for C_3 plants; Pfündel 1998). The fluorescence values are presented in relative units

Schansker et al. 2003; Strasser et al. 2007, 2010; Chen et al. 2012).

Understanding the thermal phase: an integrated view

Our current detailed analysis of different theories explaining the O–J–I–P transient led us to favor the interpretation of the thermal phase based on the theory of Duysens and Sweers in its modified version over the alternative theories (see Table 1). In agreement with the modified version of Duysens and Sweers Theory, even under saturating light conditions, there is no separation between the photochemical phase and the thermal phase, in the sense that the photoreduction of Q_A takes place gradually during the entire O–J–I–P transient, as the fluorescence rises from F_0 to F_M. Thus, we believe that during the photochemical phase, the O–J phase, only a fraction of PSII centers are closed, PSII centers being in one of the three states $Q_A^-Q_B$, $Q_A^-Q_B^-$ or Q_AQ_B at the J level (see e.g., Stirbet et al. 1998; Zhu et al. 2005), while during the thermal phase J–I–P(= M) the PSII centers continue to gradually close in parallel with PQ-pool reduction, all active PSII centers being closed only when the fluorescence yield reaches its maximum F_M. The temperature sensitivity of the J–I–P phase, the thermal phase, is due to the initiation of PQH_2/PQ exchange reactions at the Q_B site, and later also at the Cyt b_6/f. Munday and Govindjee (1969a, b) (see also Schansker et al. 2005) showed that although the photoactivity of PSII plays the major role during the photochemical phase of the fast FI, both PSII and PSI reactions influence the variable fluorescence during the thermal phase, especially the I–P rise:

(1) The inflection point I is attained when the reduction of the PQ-pool by PSII, and its oxidation by Cyt b_6/f via PSI, reach(es) a transient equilibration. At this stage of FI the rate of PQ-pool oxidation is close to its maximum, as the electron carriers beyond Cyt b_6/f are in oxidized state (Schansker et al. 2005; Antal and Rubin 2008).
(2) The length of the plateau (and/or dip), observed usually at the beginning of the I–P phase, depends on the size of PSI pool of electron acceptors, which are reduced during this part of the transient, in agreement with the experimental results demonstrating that after treatment with DBMIB, an inhibitor of plastoquinol reoxidation at Cyt b_6/f level, the fluorescence yield increases at the I step to the maximum P level (Schansker et al. 2006).
(3) The fluorescence maximum yield is achieved when the electron acceptors of PSI are completely reduced, due to a temporal inactivation of FNR (Schansker et al. 2005, 2006; also see Munday and Govindjee 1969a, b).

We note that PSI activity was also suggested to influence the fast FI measured under low, sub-saturating light intensity, when the fluorescence transient curve shows a characteristic biphasic OIDP shape: the fluorescence increases from its minimum value to a relatively low I level, followed by a plateau (pl; or a dip D), and it finally rises for a second time to its maximum level. Rappaport et al. (2007) have shown that in isolated thylakoids, which lack electron acceptors of PSI (i.e., ferredoxin Fd), the plateau is less pronounced than in leaves. Therefore, this plateau may be due to the retardation in the reduction of the PSII acceptor side during the reduction of PSI electron acceptors; further, the fast fluorescence rise from the pl level to the P level was associated with the reoxidation of PSI acceptors in parallel with PQ-pool reduction.

As we stated earlier, the in vivo Chl *a* fluorescence yield is influenced by a number of components/factors, and some of them affect the thermal phase in parallel with Q_A quenching. For example, a transient accumulation of $P680^+$ was proposed to be at the origin of the dip, observed after the J level, and measured at very high light intensities (see Fig. 5) (Lazár 2003; Schansker et al. 2011). Also factors such as the redox state of the PQ-pool, PSII connectivity, PSII and PQ-pool size heterogeneity, and alternative ET pathways have been shown to modulate the thermal phase. Of special interest are the factors influencing the PQH_2/PQ exchange reactions, such as the local pH or transmembrane proton gradient (ΔpH) (Wraight and Crofts 1970), and the local electric field or membrane potential (ΔΨ) (proposed by Diner and Joliot 1976). However, the precise influence of ΔΨ on FI is still not clear, being a matter of debate (see also Lazár and Schansker 2009). During the photogeneration of ΔΨ, several processes are observed: (a) the formation of local electric fields in PSII and PSI due to charge separation; (b) the formation of ΔpH between the stroma and the lumen, and (c) the subsequent movement of secondary ions across the thylakoid membrane. Relevant possible effects of ΔΨ on the primary events of PSII are listed below:

(1) ΔΨ can affect equilibrium between the excited states of chlorophylls [LHC + core + P680]* and the radical pair [$P680^+Phe^-$] in its low-dielectric protein environment, modifying the rate constant of the primary radical pair formation and its radiative recombination. Dau and Sauer (1992) had proposed this hypothesis based on picosecond florescence decay data analyzed with an RRP model.
(2) ΔΨ may produce a protein conformational change modifying directly the fluorescence yield emitted by Chl antenna.

(3) $\Delta\Psi$ may affect the rates of PQH_2 reoxidation and PQ reduction, thus indirectly influencing Q_A reoxidation (Graan and Ort 1983; Belyaeva et al. 2003).

Some of the models of the fast FI have included effects of $\Delta\Psi$ on the chlorophyll fluorescence yield: Pospíšil and Dau (2002) assumed a fluorescence stimulation by $\Delta\Psi$ during the J–I rise, while Vredenberg and his coworkers (Bulychev and Vredenberg 1999, 2001; Vredenberg and Bulychev 2002, 2003; Vredenberg and Prášil 2009; Vredenberg 2011) suggested that the I–P phase is the result of $\Delta\Psi$ induced by CET-PSI activity. However, we consider both these theories to be untenable. Still, it seems possible that these mechanisms may act in parallel with Q_A reduction, inducing alterations in the fast FI curve. Light-induced structural changes at the protein level during the fast FI that influence the fluorescence yield have also been proposed (Bradbury and Baker 1983; Moise and Moya 2004a, b; Schansker et al. 2011).

Andrew Rubin and coworkers have proposed a mathematical model of the fast FI in which $\Delta\Psi$ modifies the rates of PQH_2 reoxidation and PQ reduction, and therefore influences indirectly Q_A reoxidation (see Belyaeva et al. 2003 and Rubin and Riznichenko 2009). In their picture, the influence of $\Delta\Psi$ is important during the thermal phase, affecting the appearance and the position of the inflection I. Indeed, Antal et al. (2011) have reported experimental data showing that in fast FI transients measured on the marine diatom *Thalassiosira weissflogii* the inflection point I is missing (or is significantly reduced), but it reappears after the membrane potential $\Delta\Psi$ is eliminated by treatment with valinomycin. Antal et al. (2011) concluded that the influence of membrane potential on light-induced fluorescence transients in *T. weissflogii* could involve changes in downregulation of PQ cycle, including proton transfer into the lumen, and/or also of charge separation/recombination reactions in PSII.

Conclusions

In this review we have critically examined theories and hypotheses concerning the origin of the variable fluorescence during the O–J–I–P(= M) transient, as measured under continuous saturating light in dark adapted samples from higher plants or algae (see Table 1). Although there is general agreement that the O–J fluorescence rise is a photochemical phase and is related mainly to the photoreduction of Q_A in the active PSII centers (either partial, or total, and also either in absence or presence of other quenchers, depending on the theory), the processes involved during the J–I–P rise (i.e., the thermal phase) are highly controversial. In agreement with the mainstream concept of Duysens and Sweers in its modified version, Q_A continues to be photoreduced during the thermal J–I–P phase, until the fluorescence reaches its maximum yield F_M; in parallel, PQ-pool is reduced, and transmembrane ΔpH starts to be built. However, a number of alternative theories have been proposed, mainly based on the original idea of Delosme (1967), in which all active PSII centers are closed at the end of the photochemical O–J phase; in these theories, the variable fluorescence during the J–I–P phase has origins other than in the reduction of Q_A. These include: disappearance of a hypothetical quencher R (Delosme 1967); reduction of a hypothetical quencher Q_2 (Joliot and Joliot 1979); reversal of PQ-pool quenching (Vernotte et al. 1979; Kramer et al. 1995; Prasil et al. 1996; Samson and Bruce 1996; Pospíšil and Dau 2002); reversal of Q_B-quenching (Schreiber et al. 1989; Samson and Bruce 1996; Vasilev and Bruce 1998; Kolber et al. 1998, Samson et al. 1999; Schreiber 2002); reduction of Phe (Vredenberg 2000); contribution of ns DLE to the variable fluorescence (Schreiber and Krieger 1996); fluorescence enhancement induced by the membrane potential $\Delta\Psi$ (Bulychev and Vredenberg 2001; Pospíšil and Dau 2002); and conformational change (Moise and Moya 2004a, b; Schansker et al. 2011). We have arrived at the conclusion that these alternative theories need much more experimental and theoretical proof in order to be convincing.

We note that the work of Rappaport and coworkers (Lavergne and Rappaport 1998; Rappaport et al. 2002, 2005; Cuni et al. 2004; Boussac et al. 2011) have been very useful for the analysis of some of the theories mentioned above. We conclude in this review that the mainstream concept, the theory of Duysens and Sweers in its modified version, is the most credible one, since many theoretical treatments and experimental results in the literature are in good agreement with it. However, we believe that some of the factors influencing the fluorescence yield that have been proposed in the alternative theories, as e.g., the membrane potential, can conceivably contribute to the modulation of the O–J–I–P transient in parallel with the reduction of Q_A, through modifications at the PSII antenna and/or at the RC, or control of the redox status of the PQ-pool (see Antal et al. 2011).

There is a caveat to our conclusion: understanding of the low value of the maximum fluorescence yield after a saturating STF (F_m^{STF}), or a train of STFs, is not yet clear in the modified version of Duysens and Sweers Theory; it needs to be studied using more sophisticated methods than done thus far. Further, Rubin and coworkers have succeeded in simulating not only the high maximum fluorescence yield F_M reached after saturating multiturnover light pulse (Lebedeva et al. 2000, 2002; Belyaeva et al. 2006), but also the low maximum fluorescence reached after a STF, F_m^{STF} (Belyaeva et al. 2006, 2008, 2011). They have

used in both the simulations the same model that includes the RRP theory for PSII photochemistry, but different rate constants of ultrafast reactions at the PSII RC level (Trissl et al. 1993). The above result is compatible with the theory of Duysens and Sweers in its modified version but it does not validate the ideas of Schreiber and Krieger (1996) since the generation of the thermal J–I–P phase, as well as the complete reduction of Q_A at the end of the O–J phase, have not yet been proved experimentally. Moreover, the quantum yield of primary PSII photochemistry (Φ_{PSII}), as predicted by this theory, is lower than that calculated from F_V/F_M, and Φ_{O_2} (Björkman and Demmig 1987; Long et al. 1993).

Although we have argued, in this review, that the modified version of Duysens and Sweers Theory explains most of the experiments, yet we believe in keeping an open mind to further development in the field. In this review, we have traced the history of the study of the “J–I–P phase of fluorescence transient”. Although we believe we see simplicity in the complexity, yet there is still complexity in our simplicity (see Poincare’s quotation at the beginning of our review).

We wonder if Barry Osmond, to whom we dedicate this review will agree that our review is indeed related to the theme “Assimilating Photosynthesis – Quintessence of Life’s Variations and Vital Inefficiencies.” We see variations and inefficiencies in our thoughts and in our writing.

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