Chapter 5

Models of Chlorophyll a Fluorescence Transients

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Summary

In this chapter we describe modeling efforts of fluorescence rise (FLR) transients over the last 20 years. During this period the complexity of the models has increased significantly. Nowadays, the more complex models consist of a combination of the Kok model for the reactions on the donor side of photosystem II (PS II), the reversible radical pair model for the primary PS II photochemistry, the two-electron gate model for electron transport on the acceptor side of PS II, reactions related to reduction and oxidation of plastoquinone (PQ) and, in some cases, of cytochrome b₅f, plastocyanin and photosystem I. In some models additional processes are considered like electric field effects and dark reactions of photosynthesis occurring in the stroma and cytosol.

The chapter begins with an introduction of topics important for the construction of a model: relevant fluorescence theories, measuring techniques, the physiology behind the FLR, the role of the integrity of the sample, enzyme kinetics and rate constants. Subsequently several published models are discussed. A major problem for many FLR models is that the fluorescence rises much faster (often by a factor 10) than experimentally observed. Possible reasons for this mismatch are discussed in the context of different models. The large majority of models is based on the postulate that the redox state of QA is the major determinant of the variable fluorescence yield. In several models P680⁺ and quenching by the PQ pool are added, but this is still insufficient to correctly model the slowest rise phase. The question is raised whether additional assumptions are needed to correctly simulate the O–J–I–P transient.

At the end of the chapter the fluorescence decrease following the initial rise is discussed. Only a few models include this part of the fluorescence transient. A flaw of these models is that they ignore the experimentally observed transient block at the acceptor side of photosystem I, limiting both electron flow and proton transport during the FLR. As a consequence, activation of photosynthesis occurs in models with considerably faster kinetics than observed experimentally.

I. Fluorescence Induction

A short introduction to Chlorophyll (Chl) a fluorescence, its relation to the function of the photosynthetic apparatus, the techniques used to measure fluorescence and a summary of processes reflected in the fluorescence rise (FLR) is given below in this section. See Govindjee et al. (1986) and Papageorgiou and Govindjee (2004) for background and further details on all aspects of Chl a fluorescence.

A. Relation Between Photosynthesis and Fluorescence

When photosynthetic samples are illuminated, excited states (ES) of pigments are formed. The excitation energy can be transferred to the reaction center (RC) pigments where the energy is used for the transfer of an electron toward the other side of the membrane (a charge separation). The transferred electrons are then transported through and along the thylakoid membrane via a chain of electron carriers in order to reduce nicotinamide adenine dinucleotide phosphate (NADP⁺). The electron transport through the membrane also drives the

Abbreviations: Chl – chlorophyll; cyt b₅f – cytochrome b₅f; ∆Ψ – transmembrane electric potential; DBMIB – 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone; DCMU (Diquor) – 3-(3′, 4′-dichlorophenyl)-1,1-dimethyleurea; ES – excited state; F₀ – minimal fluorescence; Fd – ferredoxin; FFI – flash fluorescence induction; FLD – fluorescence decrease; FLI – fluorescence induction; FLR – fluorescence rise; Fₚ – maximal fluorescence; FNR – ferredoxin-NADP⁺-reductase; F₆₈₀ – plateau in the FLR measured upon exposure to low intensity of exciting light; FR – far-red; FRR – fast repetition rate; Fᵥ – variable fluorescence; LHC II – light harvesting antenna of PS II; O, K, J (I₁), I (I₂), D, P (M, P₀), S (S₁, S₂), M (M₁, M₂), T – particular steps of the FLI; OEC – oxygen evolving complex; MV – methylviologen (1,1′-dimethyl-4,4′-bipyrindium-dichloride); p – parameter of energetic connectivity; P680 – PS II electron donor; P700 – PS I electron donor; PAP – pump and probe; PC – plastocyanin; PDP – pump during probe; Pheo – phaeophytin, the primary PS II electron acceptor; PQ – plastoquinone; PQH₂ – plastoquinol (reduced and protonated plastoquinone); PS I – photosystem I; PS II – photosystem II; QA – the primary quinone PS II electron acceptor; QB – the secondary quinone PS II electron acceptor; qE – energy dependent non-photochemical fluorescence quenching; RC – reaction center; RRP – reversible radical pair; SFK – structure, function and kinetics; S states – redox states of OEC (i = 0, 1, 2, 3); TEG – two-electron gate; TST – three state trapping; V(t) – relative variable fluorescence; Y₂ – tyrosine 161
formation of a transmembrane proton gradient (difference in proton concentrations). The proton gradient in turn drives the formation of adenosine triphosphate (ATP). The formation of ES does not always lead to a stable charge separation and subsequent electron transport. Even if the primary quinone electron acceptor of photosystem II (PS II), QA, is in the oxidized state there is a small probability that the ES can be deactivated as fluorescence or heat emission. If QA is in the reduced state this probability increases due to a longer lifetime of the ES. Fluorescence is a radiative conversion of the (usually) first singlet ES of a molecule to its ground state. In addition to fluorescence emission, the excited singlet state can be deactivated within a molecule by inter-system crossing to the triplet state or by radiationless internal conversion to the ground state by means of heat dissipation. The quantum yield of Chl fluorescence in solution is about 20–35% (Förster and Livingston, 1952; Latimer et al., 1956), but in actively photosynthesizing samples it is only about 2% (Trissl et al., 1993), mainly because of competitive quenching by photochemistry. For overviews see Ke (2001) and Papageorgiou and Govindjee (2004).

B. Summary of Used Fluorescence Techniques

Since many techniques have been used to measure Chl α fluorescence transients, a summary of the techniques and the used nomenclature is provided here first. The Chl α fluorescence transient (induction, FLI) represents the time course of the fluorescence intensity emitted by a photosynthetic sample on a sudden transition from darkness to (usually) continuous illumination. FLI consists of a fast FLR followed by a slower fluorescence decrease (FLD). The rise and subsequent decline of the fluorescence intensity on a dark to light transition were first observed by Kautsky and Hirsch (1931). As a reference to this initial observation, the FLI is often called the Kautsky effect. In one form or another, the FLI is used in almost every laboratory dealing with photosynthesis research. The measurement of Chl α fluorescence is attractive because it is non-invasive, fast and easy, and above all it provides access to a range of processes that play a role in photosynthetic energy conversion (Govindjee, 1995).

In a dark-adapted photosynthetic sample, the acceptor side of PS II is normally predominantly in the oxidized state. In this state, all RCs are called open. This state is associated with a low fluorescence intensity called minimal fluorescence, F0, and denoted as O (for origin; see Fig. 5.1). On illumination, electrons are pumped by PS II into the electron transport chain and the acceptor side of PS II becomes more reduced. As a consequence the efficiency of photochemistry declines, the lifetime of the ES increases and the fluorescence intensity goes up. After a few hundreds of milliseconds of illumination the fluorescence intensity reaches a maximal level, Fm, denoted as P (for peak), or M (for maximum) on the transient curve (see Fig. 5.1). At this level all the RCs are closed (photochemistry is zero). When the intensity is not saturating, the use of the term P (or Fp) for the maximum of the FLI curve is more appropriate. The difference between FM (FP) and F0 is called variable fluorescence, Fv.

The intensity of the exciting light does not only determine whether the FM is reached or not, but it also determines the overall shape of the FLR. If FLRs are measured using low intensities of exciting light (50–100 µmol photons m−2 s−1) the FLR lasts for several seconds and only one step can be distinguished between O and P, called plateau (denoted as Fp; Forbush and Kok, 1968). As discussed by Lavergne and Braintais (1996), the fluorescence rise to Fp is determined by a mixture of contributing processes: closure of QA non-reducing centers, changes in the fluorescence intensity due to S-state transitions of the oxygen evolving complex (OEC), equilibration between QA and the secondary quinone electron acceptor, Qb, in Qb reducing (active) RCs and, finally, a slight “actinic effect” (some Qb accumulation in active RCs in excess of the equilibrium; Hsu, 1993; Tomek et al., 2003; Schansker and Strasser, 2005).

At low light intensities in this type of experiments the excitation rate is low enough to detect the whole FLR with a fluorometer equipped with a mechanical shutter that fully opens in about 1–2 ms. At somewhat higher excitation intensities, the fluorescence transient begins to show its multi-step kinetics although this was initially obscured by the limited time resolution of the shuttered systems that were used for the early
measurements. For such transients, Munday and Govindjee (1969) introduced the steps I (inflection) and D (dip). The dip observed in the published transients (e.g. Kautsky et al., 1960) is probably due to the partial reduction of the plastoquinone (PQ) pool at the start of the measurement reducing, initially, electron flow through PS II without much of an effect on PS I activity. The presence of the dip allows the observation of what is now called the J step (see below) even with a shuttered system. In this case the FLR reaches its maximum level within about 200 ms and two steps appear between \( F_0 \) and \( F_M \), which were first denoted as \( I_1 \) and \( I_2 \) (measured with a pulse-amplitude modulation PAM fluorometer, Neubauer and Schreiber, 1987; Schreiber and Neubauer, 1987; Fig. 5.1C) and later as J and I, respectively (measured with a PEA fluorometer, Strasser and Govindjee, 1991, 1992; Fig. 5.1A). The equivalence between the J

![Diagram](image-url)

**Fig. 5.1.** Measured Chl \( \alpha \) fluorescence transients and transmission changes at 820 nm (\( I_{520} \)) beginning from a dark-adapted state. Panel A – FLR measured with a pea leaf (curve a), spinach thylakoids (curve b) and PS II membranes (curve c) and simultaneously measured \( I_{520} \) signal with the pea leaf (curve d). Minimal fluorescence (O level) of all the curves was normalized to 1. Panel B – FLRs measured with a control pea leaf (curve a), DCMU-treated pea leaf (200 \( \mu \)M, 14 h; curve b), high temperature treated barley leaf (47 °C, 5 min; curve c) and lichen thalli of *Umbilicaria hirsuta* (curve d). Minimal fluorescence (O level) of curve a was normalized to unity. Measurements were made with a PEA fluorometer upon illumination with red light of 1,800 \( \mu \)mol photons m\(^{-2}\) s\(^{-1}\) (panel A, curves a and d and panel B, curve d), 3,000 \( \mu \)mol photons m\(^{-2}\) s\(^{-1}\) (panel A, curve b and panel B, curves a and b) and 3,400 \( \mu \)mol photons m\(^{-2}\) s\(^{-1}\) (panel B, curve c) and with a laboratory-built fluorometer upon illumination with 3,500 \( \mu \)mol photons m\(^{-2}\) s\(^{-1}\) of red light (panel A, curve c). All curves are shown on a logarithmic time-axis. For description of H and G steps, see text. Panel C – FLR measured with a tobacco leaf using a PAM fluorometer upon illumination with 9,000 \( \mu \)mol photons m\(^{-2}\) s\(^{-1}\) of white light. The same curve is presented on a logarithmic time-axis in the inset. Minimal fluorescence (O level) was normalized to unity. The nomenclature used here is that of U. Schreiber (see text). Panel D – FLI measured with two different pea leaves using a PAM fluorometer upon illumination with 40 \( \mu \)mol photons m\(^{-2}\) s\(^{-1}\) of red light. Curve a is shifted to the right by 50 s. Minimal fluorescence (O level) of both the curves was normalized to unity. Data were taken from Lazár (1999) – both curves of panel D, Pospíšil and Dau (2000) – curve c of panel A, Tóth et al. (2005) – curves a and b of panel B, Boisvert et al. (2006) – curve b of panel A, Ilik et al. (2006) – curves a and d of panel A and curve d of panel B, Lazár (2006) – curve c of panel B and the curve of panel C.
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and I₁ steps and the I and I₂ steps was later established by Strasser et al. (1995). As the O–J–I–P notation is more commonly used in the literature, we shall use it in this chapter. The J and I steps measured using high intensity exciting light usually appear at about 2–3 and 20–30 ms, respectively (Fig. 5.1A). The shape of the FLR depends on the intactness of the system. During isolation of thylakoid membranes, ferredoxin (Fd) is lost from the acceptor side of PS I (Satoh, 1981) and fluorescence transients with a less pronounced I step are detected (Pospíšil and Dau, 2002; Joly et al., 2005; Boisvert et al., 2006; Fig. 5.1A). The FLR measured using PS II membranes does not contain an I step at all (Pospíšil and Dau, 2000, 2002; Heredia and De Las Rivas, 2003; Beauchemin et al., 2007; Fig. 5.1A) since such membranes only consist of PS II and a small PQ pool. In addition, in some organisms (foraminifers, zooxanthellae, lichens and some algae) an additional peak is observed beyond the P step even under standard conditions (Tsimilli-Michael et al., 1998; Ilík et al., 2006; Fig. 5.1B). However, Tsimilli-Michael et al. (1998) introduced a new nomenclature for these organisms calling P and the additional peak: H and G, respectively. In these organisms the fast fluorescence decline following P is due to a fast activation of electron transport at the acceptor side of PS I (Ilík et al., 2006). Further, high temperature stress of photosynthetic samples unveils a new step, K, occurring after 300–400 µs of illumination in the FLR (Guissé et al., 1995; Lazár and Ilík, 1997; Fig. 5.1B).

As the relative amplitude of the O–J phase and its initial slope are strongly dependent on the intensity of exciting light, the O–J phase is called the photochemical phase of the FLR (Delosme, 1967; Neubauer and Schreiber, 1987; Strasser et al., 1995). The J–I–P phase of the FLR on the other hand is called the thermal phase of the FLR (Delosme, 1967; Neubauer and Schreiber, 1987) because its kinetics is more sensitive to the temperature of the sample (within physiological range).

Once the maximum of the FLR curve is reached, fluorescence slowly decreases through the S and M (note: the same notation is used here as for a maximum M of the FLR) steps to the steady-state or terminal level T (Papageorgiou and Govindjee, 1968; Fig. 5.1D), which is reached after several minutes of illumination. Additional local maxima can be present in the FLD and then the steps are denoted as P–S₁–M₁–S₂–M₂–T (Yamagishi et al., 1978; Govindjee and Satoh, 1986; Fig. 5.1D) and under some conditions complex damped oscillations can be measured (Walker et al., 1983; Sivak and Walker, 1985).

The FLR transients mentioned thus far were all measured upon continuous illumination where PS II turns over many times and a large number of electrons are transported along the electron transport chain. To simplify the FLR, 3-(3', 4'-dichlorophenyl)-1,1-dimethylurea (DCMU, also referred to as “Diuron”) is often used. As DCMU binds to the Q₉ pocket of the D1 protein of PS II (Oettmeier and Soll, 1983; Trebst and Draber, 1986; Shigematsu et al., 1989) and thus blocks electron transport beyond QA, only a single stable charge separation can occur. As a consequence, the FLR consists of a single rise phase that strongly depends on the light intensity and at moderate to high light intensities the maximum fluorescence level is reached at about the same time as the J step in uninhibited samples (Fig. 5.1B).

A complete reduction of QA can be obtained not only by application of DCMU but also by application of a short (about 50 µs) but very intense flash of light during which the fluorescence is detected. This technique is referred to as “pump during probe” technique (PDP, Olson et al., 1996) or “flash fluorescence induction” (FFI, Nedbal et al., 1999). The shape of the PDP (FFI) transients is the same as DCMU–FLR but the rise kinetics is complete within a much shorter time. Due to the very high light intensities used, the reduction of all QA molecules is much faster than the rate of forward electron transport towards QB.

All techniques mentioned above are based on the detection of fluorescence during continuous excitation (either single or multiple turnover) of different durations. However, techniques that are based on light flashes have also been developed. In the “fast repetition rate” (FRR) technique (Kolber et al., 1998), up to 120 short (0.6–0.8 µs), sub-saturating flashes, called flashlets, are applied with a given time interval (1–2 µs) between the flashlets to produce a saturating single-turnover flash. When about 3,000
flashlets of 0.8 μs duration are applied, a multiple turnover of RCs takes place, and O–J–I–P-like FLRs are generated (Kolber et al., 1998). By changing the interval between flashlets, the effective light intensity can be modulated. Comparing the FRR transients of Kolber et al. (1998) and continuous light induced O–J–I–P transients, differences in the form are observed. Especially the slower rise phases of the FLRs (J–I and I–P) are strongly limited by dark reactions. At high intensities of light the excitation rate is much higher than the exchange of doubly reduced cytochromes of light the excitation rate is much higher than the exchange of doubly reduced cytochromes. At high intensities of light the excitation rate is much higher than the exchange of doubly reduced cytochromes.

Continuous light induced FLRs are generated (Kolber et al., 1998). By changing the interval between flashlets, the effective light intensity can be modulated. Comparing the FRR transients of Kolber et al. (1998) and continuous light induced O–J–I–P transients, differences in the form are observed. Especially the slower rise phases of the FLRs (J–I and I–P) are strongly limited by dark reactions. At high intensities of light the excitation rate is much higher than the exchange of doubly reduced cytochromes.

C. Summary of Processes Reflected in the Fluorescence Rise

Many researchers have attempted to characterize the mechanisms underlying the different phases of the FLR. This has resulted in a large number of proposals for the processes involved. In Table 5.1, a summary of the processes suggested to define each of the rise phases is given. For a more detailed description of the proposed mechanisms.

Table 5.1. Processes suggested in the literature to be involved in the O–J–I–P FLR. The table lists processes revealed from experiments with whole leaves, thylakoids and PS II membranes and by using different chemicals. Processes denoted in italics were considered in modeling of the FLR in particular studies (see also Lazár, 2006).

<table>
<thead>
<tr>
<th>Photochemical phase (O–J)</th>
<th>Thermal phase (J–I–P)</th>
</tr>
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<tbody>
<tr>
<td>Accumulation of only $Q_A^-$ from the $Q_B$ reducing PS II and the $Q_B$ non-reducing PS II</td>
<td>Accumulation of $Q_B^{(2–)}$ in addition to $Q_A^-$</td>
</tr>
<tr>
<td>The donor side of PS II</td>
<td>Protonation of $Q_B^-$</td>
</tr>
<tr>
<td>Excitation energy transfer</td>
<td>The donor side of PS II</td>
</tr>
<tr>
<td>Transmembrane electric potential</td>
<td>Transmembrane electric potential</td>
</tr>
<tr>
<td>Recombination between PS II electron acceptors and donors</td>
<td>Heterogeneity in the rate of PQ pool reduction</td>
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<tr>
<td>Electron transport through the inactive branch in PS II</td>
<td>Fluorescence quenching by oxidized PQ</td>
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<td>Electron transport reactions beyond PS II</td>
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<td>Light gradient within a sample</td>
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<td>Cyt b55</td>
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<td>Fluorescence quenching in CP43</td>
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<td></td>
<td>Fluorescence coming from PS I</td>
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<td></td>
<td>Reduction of the Q2 component</td>
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<td>Changes in yield of recombination</td>
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<td></td>
<td>Fluorescence</td>
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</tbody>
</table>
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see Lazár (2006). Note that the processes written in italics were considered in the modeling of the FLR in particular published studies. Readers who are interested in further experimental studies on the physiology and kinetics of the FLI may consult reviews related to various aspects of the FLI: general aspects of variable fluorescence (Krause and Weis, 1991; Dau, 1994; Govindjee, 1995), the complete fluorescence transient (Papageorgiou, 1975; Briantais et al., 1986; Govindjee and Satoh, 1986; Papageorgiou et al., 2007), the O–J–I–P-transient (Lazár, 1999, 2006; Schreiber, 2004; Strasser et al., 2004) and PS II heterogeneity (Black et al., 1986; Govindjee, 1990; Lavergne and Briantais, 1996; Samson et al., 1999). References to additional papers, not covered in these reviews, are included in this chapter.

II. Approaches and Assumptions in the Modeling of the Fluorescence Rise

Many models of the FLR have been published, different in their structures, approaches and assumptions. This section summarizes these aspects of the modeling of the FLR.

A. Why Model?

On a dark-to-light transition, the kinetics of the FLR first follows the reduction of the PS II acceptor side, subsequently the reduction of the PQ pool and finally the reduction of the acceptor side of PS I. In addition, the FLR is affected by PS II heterogeneity and modulation of the fluorescence intensity by other effects, e.g. S-state transitions of OEC, connectivity between PS II antennae, and electric field effects, among others. A model that would allow a perfect fit of the FLR, obtained in a single 1-s measurement, would generate an enormous amount of information on the stoichiometry and functionality of the photosynthetic apparatus. However, the complexity of the system that has to be simulated and the disagreements with respect to the interpretation of the different kinetic phases of the fluorescence transient has kept us far from a perfect model. In the absence of a model that can simulate all known experimental observations, simulations can fulfill another role. In silico, parameters can be varied that are difficult to change in leaves. By showing how the form of the transient changes in response to changes in certain parameters may help experimentalists to interpret their measurements, suggesting which parameters may be important and which can probably be ignored.

In this respect, it is important to keep in mind that already a qualitative agreement between experiment and theory is a useful goal in the case of modeling of the FLR. The FLI (FLR) is a manifestation of a very complex biological system and therefore hard to describe correctly and comprehensively – this is quite different from the modeling of technical systems that can be described correctly and where a quantitative agreement between experiments and theory is strictly required.

Modeling can be approached in two ways: fitting or simulation. In the case of a fit, a mathematical routine searches for values of model parameters to obtain the best agreement between theoretical and experimental curves. The routine automatically fits the model parameters for the best agreement, hiding the fitting procedure from the modeler’s attention. A problem with such fitting is, however, that there is often more than one theoretical solution for the same transient, because a change in the value of one parameter can be compensated by a change in another parameter (Baake and Schlöder, 1992). Thus, the question of the uniqueness of obtained model parameters is of concern. In order to find the best fit, characterized by a global minimum of a so-called criterion function, not by a local minimum, very sophisticated mathematical routines must be used (so-called genetic algorithms). However, even if the best fit is found, the obtained values of parameters need not agree with values known from other sources (e.g. Strasser and Stirbet, 2001). Therefore, the researcher still has to judge if the obtained results are really meaningful.

Simulations, on the other hand, use fixed or pre-set values for the parameters, e.g. those from literature. The generated simulated curve is subsequently compared with experimental data obtained under the same conditions. Normally no absolute agreement between experimental and simulated transient is obtained. Instead, changes in the form of the simulated transient are studied as a function of the value of a model parameter. This way the simulation procedure approaches the non-automatic, manual fitting procedure. An
advantage of simulations is that it is possible to quantify how much model parameters affect a selected property of the simulated curve (e.g. the $F_M$-value) by means of an additional, so-called control analysis (e.g. Lazár et al., 2005a; see also Chapter 15 by Ian E. Woodrow in this book).

For modeling, one can use either simpler analytical functions or more complex mathematics based on known structures, functions and kinetics (SFK). In the first case, the FLR is described by a sum of usually exponential and sigmoidal functions, which simulate or fit the experimental FLR. For the case of e.g. the DCMU–FLR this approach enables the determination of the PS II $\alpha/\beta/\gamma/\delta$ heterogeneity related to the antenna size and the energetic connectivity (Lazár et al., 2001). For the case of the non-DCMU–FLR, the analytical functions can be used for a quantitative description of the FLR and parameters derived from it (Pospíšil and Dau, 2002), but partially also for the determination of some rate constants having molecular meaning (Vredenberg, 2008a; see Chapter 6 by Wim Vredenberg and Ondřej Prášil in this book). However, even if the sum of the analytical expressions can describe the FLR very well, the functions generally do not say too much about the molecular basis of the FLR. This is in contrast to the second case when models based on known SFK to model the FLR are used. Using this approach, it is possible to determine contributions of particular reactions to particular features of the FLR. Both these approaches are described and discussed in Section III.

B. Why Measure and Simulate the Fluorescence Rise?

Rappaport et al. (2007) recently wrote that it is better to measure fluorescence transients at a low intensity of excitation light, because low light intensities are more physiological. The authors could have taken this argument one step further, if they had written that it would have been better not to measure induction transients at all, since pure dark-to-light transitions are extremely rare in nature. Why do we then invest so much energy in the study of fluorescence transients obtained under such non-physiological conditions? The advantage of dark-to-light transitions is the well-defined dark-adapted state. We know quite well what the redox-state of the manganese cluster of the OEC, $Q_A$ and the PQ pool are in the dark. The use of the high-intensity excitation light of about 3,000 $\mu$mol photons $m^{-2} s^{-1}$ also has clear advantages: an excitation rate of about once per 200–300 $\mu$s makes it possible to detect the reduction of the acceptor side of PS II, the reduction of the PQ pool and the reduction of the acceptor side of PS I (Lazár and Pospíšil, 1999). At more physiological, lower intensities of light, the excitation rate of PS II is so low that only the reduction of the acceptor side of PS I can still be detected, as noted also by Rappaport et al. (2007). Pospíšil and Dau (2002) and Boisvert et al. (2006) demonstrated that the FLR can be approximated by three exponentials. We do not believe that the complete O–J–I–P FLR can be well simulated using only three exponentials, but it shows that the different phases of the transient measured with high light intensities are kinetically well separated. As noted by Tóth et al. (2007b) the kinetic separation between the reduction of the PQ pool and the reduction of the PS I acceptor side disappears if the PQ pool was already partially reduced at the start of the measurement. Up to the re-oxidation of reduced PQH$_2$ by the cyt b$_\delta$f complex each subsequent step is slower than its predecessor. As a consequence, a lot of stoichiometric information on the whole photosynthetic electron transport chain can be obtained. Also from a simulation point of view, measurements at high light intensities have clear advantages. Since more reactions taking place in the electron transport chain have an effect on the form of the transient, there is less uncertainty with respect to the correctness of the simulation.

C. Relationship Between the Origin of Fluorescence and the Model Structure

According to our current understanding, at room temperature and under physiological conditions variable fluorescence originates from PS II (for reviews see Krause and Weis, 1991; Dau, 1994; Govindjee, 1995). Therefore, most models of the FLR describe electron transport reactions occurring in PS II and the PQ pool (Stirbet et al., 1998; Lazár, 2003; Zhu et al., 2005). However, fluorescence measurements on intact systems show a clear effect on the FLR of electron transport through PS I (Kautsky et al., 1960; Munday and Govindjee, 1969; Satoh and Katoh, 1981; Hansen et al., 1991; Schansker et al., 2003, 2005). Therefore, some models consider also electron
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transport through PS I (Lebedeva et al., 2002; Kroon and Thoms, 2006; Laisk et al., 2006; D. Lazár, 2007, unpublished; see Section III.B.5).

In addition to the effect of electron transport through PS I, fluorescence emission emitted by PS I can be considered. Schreiber et al. (1989) interpreted the parallelism between the I–P phase of the FLR and the absorbance decline of the 820 nm signal, representing the re-reduction of oxidized P700 (PS I electron donor), and oxidized plastocyanin (PC), to indicate that the I–P phase represented variable PS I fluorescence. However, in general, fluorescence originating in PS I is assumed not to be variable, i.e., it has a constant level during the FLR and therefore fluorescence emitted by PS I contributes only to the F0 level. Trissl et al. (1993) calculated, on the basis of a trapping time constant of 110 ps, that PS I fluorescence yield is about 20% of that of PS II. A contribution of about 25% PS I fluorescence to the overall fluorescence yield (detected from 650 to 800 nm) was found from a deconvolution of the emission spectrum of an intact leaf measured at room temperature (Stahl et al., 1989). Other publications have shown that this contribution is wavelength dependent. At emission wavelengths greater than 700 nm the contribution of the PS I fluorescence to F0 can be about 30% for C3 plants (Genty et al., 1990; Pfündel, 1998; Gilmore et al., 2000; Franck et al., 2002) and 50–55% for C4 plants (Genty et al., 1990; Pfündel, 1998).

The lack of variable PS I fluorescence in leaves at room temperature is supported by a comparison of control leaves and leaves inhibited with either DCMU or 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB) (Schansker et al., 2005; Tóth et al., 2005). As indicated by 820 nm transmission measurements (I820), in the uninhibited leaves, PC, P700 and Fd are reduced at FM whereas in the presence of the inhibitors PC and P700 are in the oxidized state. However, the FM value is the same in both cases. This confirms earlier observations that the PS I fluorescence yield does not depend on the PS I redox state. This observation is frequently explained on the basis of two assumptions: (i) P700+ is a non-photochemical quencher of excitation energy (Butler and Kitajama, 1974) and (ii) in PS I the efficiency of the utilization of excitation energy for primary charge separation is approximately the same as the efficiency of excitation energy quenching by P700+. In other words, it is assumed that the fluorescence emitted by PS I is independent of the redox state of P700. However, a very detailed SFK-based model of PS I alone (D. Lazár, 2006, unpublished) shows an FLR, but this PS I variable fluorescence is probably very small compared with PS II variable fluorescence.

D. Basic Types of Photosystem II Models

Two basic models of PS II exist which are combined or extended in various ways in modeling of the FLR. One is the so-called two-electron gate (TEG) model. It describes electron transport between QA and QB and the fact that QB, unlike QA, is a two electron acceptor (Bouges-Bocquet, 1973; Velthuys and Amesz, 1974; Crofts and Wraight, 1983; Fig. 5.2; Table 5.2). This model was mainly used for modeling of FLRs measured

![Fig. 5.2. A scheme of the TEG model. The mechanism of the two electron gating is represented by the middle line of the reactions. Reactions marked by dashed arrows and reactions upward and downward of the dashed arrows describe reversible exchange of doubly reduced QB (after its protonation which is implicitly assumed) with PQ molecule from the PQ pool and reversible oxidation of reduced and protonated PQ molecule (PQH2). For rate constants see Table 5.2.](image-url)
on thylakoid membranes illuminated by a low intensity of exciting light characterized by the O–I–P steps (see Section III.B.2). However, extended versions of the TEG model were also used for modeling of the O–J–I–P transient (see Section III.B.3). When the TEG model is used for the modeling of the FLR, the fluorescence signal is assumed to be proportional to the amount of reduced $Q_A$ (see Section II.F).

The second basic model is the reversible radical pair (RRP) model (Breton, 1983; Van Grondelle, 1985; Schatz et al., 1987, 1988; Leibl et al., 1989; Roelofs et al., 1992; Fig. 5.3; Table 5.2), which describes the formation of ES and primary photochemistry (charge separation, recombination, and stabilization) in “open” ($Q_A$) and “closed” ($Q_A^-$) PS II, i.e., it describes electron transport from a PS II electron donor, P680, via the primary electron acceptor pheophytin (Pheo) to $Q_A$. The original RRP model was suggested for interpretation of pico- and nanosecond-scale transients following excitation by a very short laser flash and, therefore, this original RRP model does not include the much slower reduction of $P680^+$ by the PS II donor side. In order to use the RRP model for the modeling of the FLR, the model must be extended involving the $P680^+$ reduction processes (see Section III.A.2). The original RRP model also assumed that all the pigments of the adjacent light harvesting antennae are in ultra-fast kinetic equilibrium with pigments of RC II and with the donor pigment, an assumption of the so-called monopartite model (see Dau, 1994). In addition, the original RRP model assumed that P680 is the primary PS II electron donor. However, later findings suggested that an accessory chlorophyll, Chl$\text{acc}$ D1, that is located between P680 and Pheo, is the primary PS II electron donor (see e.g. Groot et al., 2005; Holzwarth et al., 2006). Therefore, the RRP model was extended to include also these facts (for more details see Chapter 3 by Rienk van Grondelle, Vladimir I. Novoderezhkin and Jan P. Dekker in this book). When the RRP model is used for the modeling of the FLR, the fluorescence signal can be assumed to be proportional to the amount of reduced Pheo and/or $Q_A$ or to the deactivation of the ES (see Section II.F). As the RRP model describes only electron transport up to $Q_A$, the RRP model alone can only be used for modeling of the DCMU–FLR (see Section III.A.2).

Neither TEG nor the RRP model includes an explicit description of PS II with respect to the electron transport properties on the PS II donor side, i.e. electron transport from tyrosine 161 ($Y_Z$) to P680 and electron transport from OEC to $Y_Z$. Therefore, to have a complete description of PS II, $Y_Z$ and OEC should also be considered. During PS II turnover, the Mn cluster of the OEC, together with $Y_Z$, cycles through five redox-states, the S-states, as described by the Kok model (Kok et al., 1970; Fig. 5.4; Table 5.2).

![Fig. 5.3. A scheme of the RRP model. The original RRP model is represented by the two lower lines of reactions, where the upper line represents reactions occurring in an open RC II ($Q_A$ is initially oxidized) and the lower line represents reactions occurring in a closed RC II ($Q_A$ is initially reduced). The reaction marked by a dashed arrow and reactions above the dashed arrow describe a reversible reduction of $P680^+$ by $Y_Z$ and a reduction of $Y_Z^-$ by OEC. $S_i$ and $k_S$ represent particular S-state and the related value of the rate constant, respectively, according to the Kok model (see Fig. 5.4). LH–P means all Chls of the adjacent light harvesting antennae of PS II (LH), which are in ultrafast kinetic equilibrium with P680 (P). For rate constants see Table 5.2.](image-url)
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Table 5.2. A summary of approximate values of the rate constants of a control sample (room temperature, no chemicals) considered in the TEG (Fig. 5.2), RRP (Fig. 5.3) and Kok’s S-state (Fig. 5.4) models. Rate constants are first order or are considered to be pseudo-first order (for the second order reactions) and all have dimensions of s$^{-1}$. Open and closed states of RC II mean states with QA initially oxidized and reduced, respectively. For more details on known ranges of rate constants and related references see e.g. Lazár (2003) and Zhu et al. (2005).

<table>
<thead>
<tr>
<th>Rate constant</th>
<th>Value, s$^{-1}$</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_L$</td>
<td>4,000</td>
<td>For 3,000 μmol photons m$^{-2}$ s$^{-1}$; rate of ES formation</td>
</tr>
<tr>
<td>$k_{II}^{+}$</td>
<td>$3 \times 10^9$</td>
<td>Charge separation in open RC II</td>
</tr>
<tr>
<td>$k_{II}^{-}$</td>
<td>$3 \times 10^8$</td>
<td>Radiative charge recombination in open RC II</td>
</tr>
<tr>
<td>$k_2$</td>
<td>$2.3 \times 10^8$</td>
<td>Charge stabilization in open RC II</td>
</tr>
<tr>
<td>$k_3$</td>
<td>$4.8 \times 10^8$</td>
<td>Charge separation in closed RC II</td>
</tr>
<tr>
<td>$k_{II}^{-}$</td>
<td>$3.4 \times 10^8$</td>
<td>Radiative charge recombination in closed RC II</td>
</tr>
<tr>
<td>$k_4$</td>
<td>$1 \times 10^8$</td>
<td>Nonradiative charge recombination in closed RC II</td>
</tr>
<tr>
<td>$k_5$</td>
<td>$5 \times 10^8$</td>
<td>Deactivation of ES (includes heat dissipation and fluorescence emission)</td>
</tr>
<tr>
<td>$k_{UU}$</td>
<td>$1 \times 10^9$</td>
<td>Energy transfer between open and closed RC IIs</td>
</tr>
<tr>
<td>$k_{-y}$</td>
<td>$4-40 \times 10^6$</td>
<td>Reduction of P680$^+$ by YZ; S-state dependent</td>
</tr>
<tr>
<td>$k_{-y}$</td>
<td>$1-7 \times 10^6$</td>
<td>Backward electron transport from P680 to YZ; S-state dependent</td>
</tr>
<tr>
<td>$k_{11}$</td>
<td>20,000</td>
<td>$S_0$→$S_1$ transition of OEC</td>
</tr>
<tr>
<td>$k_{12}$</td>
<td>10,000</td>
<td>$S_1$→$S_2$ transition of OEC</td>
</tr>
<tr>
<td>$k_{23}$</td>
<td>3,330</td>
<td>$S_2$→$S_3$ transition of OEC</td>
</tr>
<tr>
<td>$k_{30}$</td>
<td>1,000</td>
<td>$S_3$→$S_0$ transition of OEC</td>
</tr>
<tr>
<td>$k_{AB1}$</td>
<td>3,500</td>
<td>Electron transport from $Q_A^-$ to $Q_B$</td>
</tr>
<tr>
<td>$k_{BA1}$</td>
<td>175</td>
<td>Backward electron transport from $Q_B$ to $Q_A$</td>
</tr>
<tr>
<td>$k_{AB2}$</td>
<td>1,750</td>
<td>Electron transport from $Q_A^-$ to $Q_B$</td>
</tr>
<tr>
<td>$k_{BA2}$</td>
<td>35</td>
<td>Backward electron transport from $Q_B^-$ to $Q_A$</td>
</tr>
<tr>
<td>$k_{B/(P/Q),ex}$</td>
<td>70–800</td>
<td>Exchange of $Q_B^-$ (includes its protonation) with oxidized PQ molecule from PQ pool</td>
</tr>
<tr>
<td>$k_{(P/Q),ex}$</td>
<td>70–800</td>
<td>Backward exchange of reduced PQ molecule (includes its deprotonation) from PQ pool with $Q_B^-$</td>
</tr>
<tr>
<td>$k_{Qox}$</td>
<td>10–500</td>
<td>Oxidation of reduced PQ pool</td>
</tr>
<tr>
<td>$k_{Qred}$</td>
<td>10–500</td>
<td>Backward reduction of oxidized PQ pool</td>
</tr>
</tbody>
</table>

Fig. 5.4. A simplified scheme of the Kok’s S-state model. $S_i$ ($i = 0$, 1, 2, 3, 4) represents the particular S-states of the manganese cluster of OEC. The $S_0$-state is assumed to be kinetically indistinguishable from the $S_0$-state. YZ is the electron donor to P680. For rate constants see Table 5.2.

The models of e.g. Lazár (2003) and Zhu et al. (2005) consist of a combination of the TEG, RRP and Kok models to simulate the FLR (see Section III.B.4).

E. Kinetics and Rate Constants

Mass action theory is routinely used in modeling of the FLR. However, a proper choice between first and second order reactions has to be made. For example, if we assume that electron transport from $Q_A^-$ to $Q_B$ in a model is irreversible, this reaction can be described as: (i) $[Q_A^+] + [Q_B] \rightarrow [Q_A] + [Q_B^-]$ or as (ii) $[Q_A] + [Q_B] \rightarrow [Q_A] [Q_B^-]$, where (i) is a second order reaction with a rate of product formation $d[Q_A]/dt = d[Q_B]/dt = \text{rate constant} \times [Q_A^-(t)] \times [Q_B](t)$ and (ii) is a
first order reaction with a rate of product formation \(d[Q_A Q_B^-]/dt = \text{rate constant} \times [Q_A^- Q_B](t)\).

From the point of view of chemical reaction kinetics, second order kinetics can be used when two components can freely move and react together in a free volume that can be used for the interaction of e.g. PQ with the \(Q_B\) pocket, PQ with cyt \(b_6\), PC with cyt \(b_6\), PC with P700, etc., but not for reactions occurring inside a protein (e.g. electron transport from \(Q_A^-\) to \(Q_B^{0^-}\)), where the components are fixed in their positions. In this case first order kinetics should be used. From a “physiological” point of view, second order kinetics in fact means that \(Q_A^-\) from one PS II can react with \(Q_B\) from any other PS II, which is of course not the case (see also Chapter 7 by Andrew Rubin and Galina Riznichenko in this book). The different kinetics also leads to different simulations of the FLR as shown by Stirbet and Strasser (1995, 1996).

Another point of which a researcher must be aware is related to the values of rate constants used in the model. The published values of rate constants present several potential problems to the modeler. In the first place, the experimental conditions used to determine the rate constant do not necessarily match the found conditions e.g. in a leaf. To give an example of this, the equilibrium constant for electron transport between \(Q_A^-\) and \(Q_B\) depends on the pH of the stroma (Robinson and Crofts, 1984). If the rate constant used in the model was determined at pH 7, but the stroma of a dark-adapted leaf is approximately at pH 7.5 (Hauser et al., 1995; Schansker and Strasser, 2005), there will be a mismatch. Also the intactness of the system used to determine the rate constants may affect their values. Rate constants based on measurements with PS II core particles may deviate from the values found in leaves. An additional problem occurs if the technique used to determine a rate constant was not precise enough and the obtained value is in reality only a rough estimate. An example of the differences between experimental material and technical limitations is presented by the list of very different values for the halftime of the particular S-state transitions collected by Razeghifard et al. (1997). Further, almost every model is a simplification and a reaction in a model may in fact consist of several partial reactions. Therefore, it is important to know which partial reaction is rate limiting for the model reaction, in order to use the appropriate value.

According to the Marcus theory of electron tunneling in biological systems (Marcus and Sutin, 1985) and its empirical simplification (Page et al., 1999, 2003), the rate constant of an electron transport reaction in a protein \((k_{at})\) depends (i) on the edge-to-edge distance \((R)\) between electron donor and acceptor, (ii) on the reorganization energy \((\lambda)\), and (iii) on the driving force of the reaction (difference in standard free energy, \(\Delta G^0\)):

\[
\log k_{at} = 15 - 0.6R - 3.1(\Delta G^0 + \lambda)^2/\lambda.
\]

(5.1)

For the origin of the numeric terms in Eq. (5.1) see Page et al. (1999, 2003). However, in RC IIs (or in thylakoid membranes in general) several processes take place that can affect this simple equation. Light-induced electron transport causes structural changes in the protein (Christophorov et al., 2000; Goushcha et al., 2000). Simultaneously, a light-induced electric field is evolving. The formation of the electric field effect can be divided into three components: (i) formation of the electric field inside PS II due to charge separation, (ii) transmembrane electric potential \((\Delta \Psi)\) caused by the transport of protons during the initial formation of a pH gradient between the stroma and the lumen and (iii) changes in \(\Delta \Psi\), caused by the subsequent movement of accompanying secondary ions across the thylakoid membrane. These processes affect the values of the rate constant for particular electron transport reactions. In the absence of experimental data giving insight in the precise effects of these processes on electron transport rates and, in addition, the complicated mathematics involved, these processes have seldom been considered in modeling of the FLR. Usually the rate constants are assumed to be constant in time or, in some cases, the effect of electric field has been considered (see Sections III.B.4 and III.B.5).

According to the RRP model, the above process (i) is manifested as a decrease of the rate constant for charge separation and an increase of the rate constant for charge recombination when \(Q_A^-\) is already (initially) reduced (Schatz et al., 1987, 1988). Similarly, a decrease of the rate constant of charge separation and an increase of the
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rate constant of charge recombination was documented for process (iii) (Dau and Sauer, 1991, 1992). The changes in the above-mentioned rate constants increase the concentration of the ES and therefore lead to an increased fluorescence yield (Dau and Sauer, 1991, 1992). But process (iii) does not affect the fluorescence yield in initially closed ($Q_A^-$) RC II (Bulychev et al., 1986; Dau and Sauer, 1991), probably because the ion-related transmembrane electric field is too weak compared to the local field induced by the electron stored on $Q_A$. However, the theory of Wim Vredenberg and Alexander Bulychev (the so-called photo-electrochemical fluorescence stimulation) predicts a fluorescence increase caused by process (iii) even if all the RC IIs are closed (Bulychev and Niyazova, 1989; Vredenberg and Bulychev, 2002; Vredenberg et al., 2006; see Chapter 6 by Wim Vredenberg and Ondřej Prášil in this book). Graan and Ort (1983) showed that during the first 200 ms of illumination valinomycin (ionophore known to short-circuit the flow of $K^+$ ions) was able to accelerate the rate of plastoquinol oxidation, whereas nigericin (supposed to act as protonophore) was ineffective. These authors therefore concluded that process (iii) and not process (ii) dominates the decrease of the rate of plastoquinol oxidation. On the other hand, process (ii) begins to dominate changes in the rate of plastoquinol oxidation after longer periods of illumination. The additive effect of $\Delta \Psi$ and pH changes agrees with the work of David Kramer and coworkers (Cruz et al., 2001; Kramer et al., 2003). It is important that process (ii) not only affects plastoquinol oxidation, but also affects electron transport from $Q_A^-$ to $Q_B$: an alkalinization of stroma causes a decrease in the apparent equilibrium constant of the electron transport reaction (Robinson and Crofts, 1984), i.e., the probability to find an electron on $Q_A$ increases (see also Belyaeva et al., 2006 and Sections III.B.4 and III.B.5). The biophysical mechanism behind all the electric field induced changes of the rate constants – direct and reverse – is a change in the $\Delta G^\circ$ of a given reaction.

For the proper use of rate constants in the modeling of the FLR, attention should also be paid to their units, which are either s$^{-1}$ or M$^{-1}$ s$^{-1}$, for the first and second order reactions, respectively. The first order rate constants range for PS II from about $10^{11}$ s$^{-1}$ (primary charge separation) to about $10^3$ s$^{-1}$ (electron transport from $Q_A$ to $Q_B$) and the value of the second order rate constant related to PS II is about $10^6$ M$^{-1}$ s$^{-1}$ (binding of a PQ molecule to the $Q_B$ pocket of PS II). To simplify and speed up calculations, a normalization is used. For the basic TEG model, the initial concentration of $Q_AQ_B$ (in the dark-adapted state) is set to 1 (instead of about 0.05 mM, typical for a thylakoid suspension of $20 \mu$g Chl ml$^{-1}$). After rescaling of the rate constants, the first order rate constants have the same values and units (s$^{-1}$) as the original values, but the second order rate constants, having much smaller values than before the normalization, now have the unit s$^{-1}$ (since PS II concentration was assumed to be unity).

F. Fluorescence and “Closed” Reaction Centers

To model the FLR, one must know which processes determine the change in the fluorescence yield between O and P. Usually, it is assumed that fluorescence is proportional to the fraction of “closed” RC II. However, what characterizes a closed RC II?

Duysens and Sweers (1963) defined as closed a RC II with $Q_A^-$. Using this approach, fluorescence is directly proportional to the amount of $Q_A^-$, but on the assumption that PS II units are energetically disconnected. This assumption leads to an exponential fluorescence rise when the sample is inhibited by DCMU (see Section III.A.1). If PS II units are energetically connected, then fluorescence is expressed by Eq. (5.5) (Lazár, 1999; see also Section III.A.1). In the case of energetically connected PS II units, the fluorescence rise measured on samples inhibited by DCMU is sigmoidal. However, an alternative explanation for the sigmoidal fluorescence rise has been suggested, based on the assumption that $Y_\Delta^+$ quenches fluorescence (Vredenberg, 2008b; Section III.A.1; see also Chapter 6).

A few years after the publication of the study of Duysens and Sweers (1963), René Delosme introduced the terms photochemical phase and thermal phase, referring to the fast and the slower rise phases of the FLR (Delosme, 1967; see Section I.B). In that paper, he assumed that most $Q_A$ was already reduced at the end of the photochemical phase (J step) and therefore the J–I–P rise had to be something else. Over the years
more observations were made that suggested that Q_A was not the only determinant of the variable fluorescence. Joliot and Joliot (1979) working with DCMU-inhibited samples introduced a second quinone Q_2 (a putative electron acceptor which needs more than one flash to be reduced in the presence of DCMU; not identical with Q_B) that together with Q_1 (= Q_A) would explain the data. Another observation that is difficult to explain is the context of the hypothesis of Duysens and Sweers (1963) is that in the presence of DCMU, 2 ms of illumination is enough to reach F_M, whereas in its absence only about 60% of the maximum fluorescence yield can be reached in 2 ms even if intensities of excitation light of 15,000 µmol photons m^-2 s^-1 are used (e.g. Neubauer and Schreiber, 1987; Schansker et al., 2006). The same is true for a single turnover flash with which only about 60% of the F_M, caused by continuous excitation, can be induced (Samson and Bruce, 1996), Samson et al. (1999) tentatively suggested that the occupation state of the Q_A site also plays a role in determining fluorescence yield; Schansker et al. (2006) have provided some experimental evidence that seems to support this interpretation. There may indeed be a second determinant of the fluorescence yield, but in the absence of credible experimental evidence we can only guess what it is and what its underlying molecular mechanism may be. Suggestions found in the literature are: recombination fluorescence (Schreiber and Krieger, 1996), the redox state of Pheo (Vredenberg, 2000), electron flow along the inactive branch of PS II (Schreiber, 2002), fluorescence quenching caused by P680+ (Butler, 1972; Bruce et al., 1997) and by oxidized PQ molecules (Vernotte et al., 1979; Kurreck et al., 2000), the quenching by the oxidized PS II donor side (Hsu, 1993; Lavergne and Leci, 1993), and Y_F (Vredenberg, 2000b) and, perhaps, a conformational change, all of which would affect the fluorescence yield.

Few modelers have explored the possibility that there is a second determinant of the fluorescence yield. The main exception, in this respect, is the so-called three state trapping (TST) model put forward by Wim Vredenberg (2000; also see Chapter 6 in this book). In the TST model, closed RC IIs are defined as having either oxidized P680 or reduced Pheo. It is a state in which ES cannot be used for photochemistry. This approach was adopted from the definition of closed RCs that was originally proposed for bacteria (Vredenberg and Duysens, 1963). Because the rate constant of excitation (related to the applied intensity of actinic light) used in the FLR experiments is much lower than the rate constants of both P680+ reduction by Y_Z and Pheo oxidation by Q_A, a closed RC II is in fact in the P680Pheo^− Q_A state. Assuming that the redox state of Pheo is a co-determinant of the variable fluorescence yield, two electrons are necessary to close a single RC II. In this model, the P680PheoQ_A state is called semi-open (also called semi-closed) state, having a fluorescence yield that is half the maximal value of the P680Pheo^− Q_A state. Strasser and Stirbet (2001) and Stirbet and Strasser (2001) have explored the approaches of Duysens and Sweers (1963) and that of Vredenberg (2000), mentioned above, and have added, as a third approach, the idea that only the redox state of Pheo determines whether an RC II is open or closed. In this case the fluorescence at time t is directly proportional to the amount of Pheo^−.

Independent of the number of determinants of the fluorescence yield, the best approach would be to define the fluorescence intensity as radiative deactivation of ES, i.e. as k_F × [ES](t) where k_F is the rate constant of fluorescence emission and ES can be formed either in RC II or in the light harvesting antennae (Baake and Schlöder, 1992; Lebedeva et al., 2002; Lazár, 2003; Zhu et al., 2005). This approach takes into account the physical origin of fluorescence as presented by the well-known Jablonski diagram (see Lakowicz, 1999).

All the above approaches assume that changes in fluorescence yield are caused only by changes in the efficiency of photochemistry via changes in the amount of closed RC IIs, whatever the “closed” means. However, in principle, changes in the fluorescence yield reflect changes in the efficiency of any processes utilizing excitation energy: in addition to photochemistry also the dissipation of ES to heat. These changes are then reflected in changes of measured fluorescence lifetime(s) (e.g. Holub et al., 2000, 2007; Moise and Moya, 2004a,b). Energy-dependent non-photochemical fluorescence quenching (qE) is e.g. interpreted to be due to a change in the rate constant for heat dissipation (Gilmore et al., 1995; Vasil’ev et al., 1998). Only in the models
of Goltsev and Yordanov (1997) and Laisk et al. (2006a) it is assumed that excitation dissipation also changes during the transient, in parallel with changes in the efficiency of photochemistry.

G. Model Formulation and Simplification

In formulating a model to simulate experimental data one always has to find a balance between the correctness of the model (it should include all “important” reactions) and the simplicity of the model (simplifying both the calculations and the interpretation of the simulated curves). There are also procedures – so-called model reductions – for the simplification of complicated models (see e.g. Riznichenko et al., 1999, 2000; Lebedeva et al., 2000; Nedbal et al., 2007; Chapter 2 by Ladislav Nedbal, Jan Červený and Henning Schmidt in this book).

The implications of different approaches to model reduction can be discussed in the context of a PS II model. We could take a model that only consists of the $S_1$-states of the OEC, P680, QA, QB and the PQ pool, assuming a homogeneous population of PS II. Depending on model simplifications, it contains different numbers of state variables (also called model forms). If all electron carriers are separate state variables, there are four $S_1$-states ($S_1$, $S_2$, $S_1$, $S_0$), two states of P680 ($P680^+$ and $P680^{+}+$), two states of QA ($QA^+$, $QA^-+$), three states of QB ($QB^+$, $QB^-$, $QB^{2-}$), and two states of the PQ pool (reduced, oxidized), that is, $4 + 2 + 2 + 3 + 2 = 13$ state variables (differential equations) describing all possible redox states in this model. However, as already discussed in Section II.E, it is not correct to consider electron carriers inside PS II as separate entities (state variables).

To make a more correct model consisting of the above-mentioned electron carriers, it is possible to define a state variable, a PS II with a particular combination of redox states of P680, QA, and QB (e.g. P680$^+$/$QA^+$), but still leaving the $S_1$-states of the OEC as separate state variables. This would mean OEC is considered separated from PS II. Also, if one considers the exchange of doubly reduced QB for an oxidized PQ molecule a second order reaction (e.g., $P680^+QA^2- + PQ^{oxidized} \leftrightarrow P680^+QA^+QB^+ + PQ^{reduced}$), then there are $2 \times 2 \times 3 = 12$ state variables describing all possible redox states of PS II plus four variables describing the four $S_1$-states plus two variables describing the redox states of the PQ pool, that is 18 state variables (differential equations) describing all possible redox states in this model.

It is however well known that OEC forms an integral part of PS II and that the $Q_B^+/\text{PQ}$ exchange occurs via two subsequent reactions ($S_1P680^+QA^2- \leftrightarrow S_1P680^+QA^+E + PQ^{reduced}$ followed by $S_1P680^+QA^+E + PQ^{oxidized} \leftrightarrow S_1P680^+QA^+QB$, where E stands for an empty QB pocket). In this case there are $4 \times 2 \times 2 \times 4$ (there are now four instead of three QB states, because the empty QB pocket is considered a separate case) = 64 state variables describing all possible redox states of electron carriers inside PS II and the OEC plus two variables describing the redox states of the PQ pool. In other words, there would be 66 variables (differential equations) that describe all possible redox states in this model.

The above discussion illustrates that models with different levels of complexity can be obtained depending on the approach (simplifications) used in formulation of these models. It is also clear that even if simplifications result in “easier” models, such models do not exactly reflect the SFK properties of PS II. Moreover, as shown in Fig. 5.5 (see Lazár and Jablonský, 2000; Lebedeva et al., 2000; Nedbal et al., 2007; Chapter 2 by Ladislav Nedbal, Jan Červený and Henning Schmidt in this book).

The implications of different approaches to model reduction can be discussed in the context of a PS II model. We could take a model that only consists of the $S_1$-states of the OEC, P680, QA, QB and the PQ pool, assuming a homogeneous population of PS II. Depending on model simplifications, it contains different numbers of state variables (also called model forms). If all electron carriers are separate state variables, there are four $S_1$-states ($S_1$, $S_2$, $S_1$, $S_0$), two states of P680 ($P680^+$ and $P680^{+}+$), two states of QA ($QA^+$, $QA^-+$), three states of QB ($QB^+$, $QB^-$, $QB^{2-}$), and two states of the PQ pool (reduced, oxidized), that is, $4 + 2 + 2 + 3 + 2 = 13$ state variables (differential equations) describing all possible redox states in this model. However, as already discussed in Section II.E, it is not correct to consider electron carriers inside PS II as separate entities (state variables).

To make a more correct model consisting of the above-mentioned electron carriers, it is possible to define a state variable, a PS II with a particular combination of redox states of P680, QA, and QB (e.g. P680$^+$/$QA^+$), but still leaving the $S_1$-states of the OEC as separate state variables. This would mean OEC is considered separated from PS II. Also, if one considers the exchange of doubly reduced QB for an oxidized PQ molecule a second order reaction (e.g., $P680^+QA^2- + PQ^{oxidized} \leftrightarrow P680^+QA^+QB^+ + PQ^{reduced}$), then there are $2 \times 2 \times 3 = 12$ state variables describing all possible redox states of PS II plus four variables describing the four $S_1$-states plus two variables describing the redox states of the PQ pool, that is 18 state variables (differential equations) describing all possible redox states in this model.

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the three models described above lead to the simulation of different FLRs (keeping the values of rate constants and initial conditions in all the models the same). The transients shown in Fig. 5.5 illustrate that the chosen approach can significantly affect the shape of simulated FLR.

### III. Particular Models for the Fluorescence Rise

This section summarizes particular models and their results, used for modeling FLR with and without DCMU.

#### A. Modeling Fluorescence Rise in DCMU Inhibited Samples

As mentioned in Section I.B, in the presence of DCMU (Diuron) electron transport beyond $Q_A$ is inhibited. This results in a steep rise of the fluorescence intensity, reaching the maximum level after about 2 ms when measured with an intensity of excitation light of 3,400 μmol photons m$^{-2}$ s$^{-1}$ (Fig. 5.1B). Reducing the intensity of excitation will increase the time needed to reach the maximum level. The description of the electron transport reactions occurring in the presence of DCMU, needed for SFK-based models, is considerably simpler than the description in its absence, and can be done using analytical functions only.

1. **Using Analytical Functions**

Analytical functions have been used to explore PS II heterogeneity (Lazár et al., 2001). The method allows an analysis of PS II antenna size heterogeneity, characterized by differences in $k_i$ and heterogeneity in energetic connectivity between PS II units characterized by differences in $p_i$ (see below). This heterogeneous behavior of PS IIs was first recognized and analyzed by Melis and Homann (1975, 1976); they based their interpretation on the observation of heterogeneity in the time course of the complementary area (area between FLR curve and a horizontal line representing the $F_M$ level). Melis and Homann (1975, 1976) distinguished two phases that were ascribed to two types of PS II reaction centers: PS IIA and PS IIB that differed in their antenna size and energetic connectivity (see e.g. Black et al., 1986 and Lavergne and Braintais, 1996 for a discussion of the literature). This analysis method was further elaborated by Sinclair and Spence (1988, 1990) and Hsu et al. (1989; see also Hsu and Lee, 1991). Sinclair and Spence (1988, 1990) identified two additional kinetic phases: $\gamma$ and $\delta$. The $\gamma$-phase has been suggested to be the same as the $Q_B$ non-reducing RC IIs, just determined by other means based on their relative contributions (Schansker and Strasser, 2005) and we cannot exclude that the $\delta$-phase reflects RC IIs that were not inhibited by DCMU. For a discussion of the DCMU-leak hypothesis, see e.g. Lavergne and Braintais (1996).

It is assumed in this approach that closed RC IIs are those with $Q_A^{-}$ (see Section II.F). Closure of RC IIs can then be described as:

$$[Q_A^{-}]_i(t) \rightarrow [Q_A^{-}]_i(t), \quad (5.2)$$

where, $i$ indicates the type of RC II ($\alpha$, $\beta$, $\gamma$ or $\delta$) and the closure of each RC II, proceeds with a separate rate constant $k_i$. This can be mathematically written as:

$$[Q_A^{-}]_i(t) = [Q_A^{-}]_i(0)1 - \exp(-k_i t), \quad (5.3)$$

where, $t$ is time and $[Q_A^{-}]_i(0)$ is the fraction of a particular type of PS II ($\Sigma [Q_A^{-}]_i(0) = 1$).

If there is no energetic connectivity between RC IIs of a given type then the relative variable fluorescence $V_i(t) = (F_i(t) - F_{0,i})/(F_{M,i} - F_{0,i})$ is proportional to the amount of $[Q_A^{-}]_i(t)$ and there is an exponential rise of fluorescence as described by Eq. (5.3). On the other hand, if RC IIs of a given type are energetically connected, the closure of the RC IIs with time causes an increase in the effective antenna size of the remaining open RC IIs, leading thus to an increase of the rate of the $Q_A$ reduction in the remaining open RC IIs. Therefore, $k_i$ is a function of time:

$$k_i(t) = k_i^0 / (1 - p_i [Q_A^{-}]_i(t)), \quad (5.4)$$

where $k_i^0$ is the initial rate constant (at $t = 0$) for the closure of a particular type of RC II (i.e., for $Q_A$ reduction) and $p_i$ is the connectivity parameter according to Joliot and Joliot (1964). A formula for $k_i(t)$ similar to Eq. (5.4) was also derived for a situation in which energetic con-
5 Modeling Fluorescence Transients

nectivity between all RC IIIs is unlimited (RC IIIs behave according to the so-called lake model; for a review see Lazár, 1999). $V_i(t)$ of the energetically connected RC IIIs is described as:

$$V_i(t) = \frac{(1-p_i)[Q_A^\lambda](t)}{(1-p_i)[Q_A^\lambda](t)}$$

which leads to a sigmoidal rise of fluorescence. Equations similar to Eq. (5.5) were derived by several authors (for a review see Lazár, 1999). An alternative explanation has been suggested by Vredenberg (2008b); in this interpretation, the sigmoidicity is caused by comparable rates of formation of the charge-stabilized state and of the release of fluorescence quenching by $Y_Z^+$ without any consideration of energetic connectivity between PS II units (see Chapter 6).

It is important to note that the approach described above is generally valid and is also used for simulations of the FLR measured without DCMU using models where fluorescence is assumed to reflect the amount of $Q_A^\lambda$ (see sections below).

2. The Application of Reversible Radical Pair Models

At first sight, the DCMU–FLR contains a feature that is sometimes misleading. At an intensity of excitation light of about 3,400 µmol photons m$^{-2}$ s$^{-1}$ the maximum fluorescence intensity is reached after 2–3 ms (Fig. 5.1B). For the used light intensity, one excitation occurs per 200–300 µs (Lazár and Pospíšil, 1999) that equates to about ten excitations (in theory ten charge separations) during the 2–3 ms interval. However, when DCMU is present, a maximum of two excitations is used for photochemistry leading to storage of only two electrons, one on Pheo and one on $Q_A^\lambda$. This discrepancy is explained on the basis of the RRP model (Fig. 5.3) if reduction of $P680^+$ by the PS II donor side is included in the model: only following a reduction of $P680^+$ by $Y_Z^+$ and a re-reduction of $Y_Z^+$ by the manganese cluster of OEC the recombination reactions are prevented and it takes, therefore, some time to reach the maximum value (Lavergne and Rappaport, 1998).

Trissl et al. (1993) were the first to apply the RRP approach to the modeling and analysis of the DCMU–FLR. However, they used the original RRP model (see Section II.D), which does not explicitly include the $P680^+$ reduction by the PS II donor side. To bypass this problem, Trissl and coworkers calculated the amount of the respective state variables using a backward (recursive) way (that is also the reason why the FLRs are shown in this study on a pseudo time base). Some numerical errors in this paper (for a discussion of these errors see Falkowski et al., 1994; Trissl, 1994) allowed Holzwarth (1993) to suggest that no useful information could be obtained on the basis of the DCMU–FLR. The RRP model was further used to explore the relationship between the fluorescence transient and energetic connectivity (Lavergne and Trissl, 1995) and PS II antenna heterogeneity (Trissl and Lavergne, 1995). Trissl and Lavergne (1995) concluded on the basis of their analysis that energetic connectivity due purely to a PS II$a$ dimer organization was less likely, but that a PS II$a$ dimer organization with limited energetic connectivity between the individual dimers would explain the results well. Vavílín et al. (1998) used the model of Lavergne and Trissl (1995) to analyze photoinhibition; they assumed that photoinhibited PS IIIs do not trap ESs for photochemistry but efficiently dissipate the absorbed light energy.

All studies mentioned above used the original RRP model and therefore did not take into account the influence of the PS II donor side on the results. Heat stress destroys the manganese cluster of OEC and thereby changes the properties of the donor side of PS II (Cheniae and Martin, 1970; Kimimura and Katoh, 1972; Enami et al., 1994; Tóth et al., 2007a). To analyze the effects of a high temperature treatment on the DCMU–FLR, the original RRP model was extended adding a description of the donor side reactions (Lazár and Pospíšil, 1999). Using this model, a fit of the experimental DCMU–FLR could be obtained without the assumption of PS II heterogeneity.

Lazár et al. (2005a) observed a nearly linear relationship between the amount of initially reduced cyt b$_{559}$ and the $F_M$ level in DCMU-inhibited thylakoid membranes. To explain this relationship the RRP model was extended to include also cyt b$_{559}$, which was assumed to accept electrons from Pheo$^-$ and donate electrons to $P680^+$ thereby enabling a well-known cyclic electron transport around PS II (Heber et al., 1979; Falkowski et al., 1986; Miyake and Yokota, 1979; Falkowski et al., 1986; Miyake and Yokota, 1979; Falkowski et al., 1979; Miyake and Yokota, 1986).
2001; Laisk et al., 2006). In the crystal structure of RC II (Kamiya and Shen, 2003; Ferreira et al., 2004; Loll et al., 2005) cyt b559 was found to be located on the Q_{B} side of the RC. Therefore, the Pheo donating electrons to cyt b559 in the model of Lazár et al. (2005a) is possibly the Pheo localized in the D2 protein, which is part of the inactive branch of PS II. The inactive branch has been suggested earlier to play a role in the FLR (Schreiber, 2002).

B. Modeling of the O–(J–)I–P Phases in Fluorescence Rise

This section summarizes particular approaches used for modeling of the FLR measured when dark-adapted samples are exposed to low (O–I–P curve) or high (O–J–I–P curve) intensity of exciting light.

1. Using Analytical Functions

Pospíšil and Dau (2000, 2002) and Boisvert et al. (2006) used a very simple approach to fit the Chl a fluorescence transients measured on PS II and thylakoid membranes. In all cases, these authors assumed that the transients could be fitted with two to three exponentials. For PS II-membranes Pospíšil and Dau (2002) argued that the O–J phase represented the reduction of Q_{A} whereas the rest of the rise (the J–P phase) was due to the reduction of the PQ pool and a concomitant loss of quenching by PQ. These two processes were assumed, for all practical purposes, to be kinetically independent. Pospíšil and Dau (2002) assumed that the third phase (the J–I phase) that occurs in thylakoid membranes was due to ΔΨ. Support for this interpretation was found in the effect of the ionophore valinomycin: it caused the reduction of the amplitude of the J–I phase

\[ \text{Boisvert et al. (2006) used the same three-exponential approach in combination with an analysis of Arrhenius plots for their analysis of the FLR, and noted complementarity between O–J and J–I amplitudes. This complementarity is a logical consequence of the fact that the FLR reflects the kinetics of a series of sequential reactions. It means that the amplitudes of the different phases of the FLR are interdependent. Therefore, in our opinion, the three-exponential analysis does not necessarily yield useful quantitative information. Rather, the fact that the three-exponential analysis works at all makes it clear that the three phases of the FLR are kinetically well separated.}

Pospíšil and Dau (2002) and Boisvert et al. (2006) used the following formula to fit the FLR:

\[
F(t) = F_{0} + A_{0-1}[1 - \exp(-k_{0-1}t)] + A_{1-2}[1 - \exp(-k_{1-2}t)] + A_{2-3}[1 - \exp(-k_{2-3}t)],
\]

where, A’s and k’s are amplitudes and rate constants, respectively, of the particular phases of the FLR. Similarly, Pospíšil and Dau (2002) fitted the FLR of PS II membranes using Eq. (5.6) but without the central product because PS II membranes do not show the J–I phase of the FLR. We note that Eq. (5.6) is a sum of pure exponentials and therefore it does not take into account the energetic connectivity between PS II units (see Section III.A.1.).

Antal and Rubin (2008) have used Eq. (5.6) without the F_{0} term for fitting not of raw curves F(t) as was done previously (Pospíšil and Dau, 2000, 2002; Boisvert et al., 2006), but of a transformed curve, F_{transformed}(t), according to the following formula:

\[
F_{\text{transformed}}(t) = [1 - F_{0} / F(t)](F_{V} / F_{M})^{-1}.
\]

Antal and Rubin (2008) derived that F_{transformed}(t) should reflect the relative amount of closed PS IIIs (relative amount of Q_{A}). However, experimental data confirming the relationship between the transformation and the amount of closed PS IIIs will be needed to make this a viable approach.

Vredenberg (2008a; Chapter 6) also uses a sum of exponentials to fit the FLR. However, the functions used by Vredenberg (2008a) partly represent the different electron transport reactions that...
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play a role in his TST model (Vredenberg, 2000; see Sections II.F and III.B.3) and therefore do not represent the type of extreme simplification that dominated the approach discussed above. The Vredenberg model of the FLR is for that reason more likely to yield meaningful information on the molecular basis of the FLR.

2. Two-electron Gate Models

The basic TEG model, as shown in Fig. 5.2, was used for the modeling of the O–I–P FLR measured in low intensity of exciting light. Renger and Schulze (1985) demonstrated for thylakoid membranes that it is only possible to fit the experimental FLR when there is an exponential decrease in the rate of exchange of doubly reduced $Q_B$ with PQ as a function of the time of illumination. Renger and Schulze (1985) suggested that the changes in the exchange rate might be due to conformational changes. A 3:1 ratio between PS II$\alpha$ and PS II$\beta$ centers, a connectivity parameter $p$ of 0.5 for PS II$\alpha$ centers, and 5.5 and 1.5 PQ molecule per PS II$\alpha$ and PS II$\beta$-center, respectively, were needed to obtain a satisfactory fit (Renger and Schulze, 1985). Hsu (1992) criticized this study by noting that the initial decrease in the rate of exchange of doubly reduced $Q_B$ with PQ as a function of the time of illumination. Hsu (1992) and Tomek et al. (2003) concluded on the basis of their TEG-model simulations that $Q_B$ reducing (=active) PS II centers contribute to the $F_{pl}$ level of the FLR. As noted by Hsu (1993), this contribution is due to the equilibrium between $Q_A$ and $Q_B$. These studies confirmed the original observation of Forbush and Kok (1968) that the dark-adaptation kinetics of the $F_{pl}$ level was biphasic and therefore presented two processes. As noted among others by Lavergne and Braintais (1996) and Tomek et al. (2003) the $F_0 - F_{pl}$ rise overestimates the amount of the $Q_B$ non-reducing PS II centers. However, as shown by Schansker and Strasser (2005), using a far-red (FR) light pre-pulse, it is possible to reduce the problem to these two components ($Q_B$ non-reducing PS II and the equilibrium between $Q_A$ and $Q_B$) of which the relative amplitudes can be determined by a kinetic analysis.

The basic TEG model was also used for modeling of the O–J–I–P FLR measured upon a high intensity of exciting light. The first of these studies was published by Stirbet and Strasser (1995) followed by other papers (Stirbet and Strasser, 1996; Strasser and Stirbet, 1998). In these studies, Alexandrina Stirbet and Reto Strasser showed how parameters like the PQ pool size, the rate constant for the re-oxidation of $Q_{PQH_2}$ and the excitation rate $k_1$ (Stirbet and Strasser, 1995, 1996) and PS II heterogeneity (Strasser and Stirbet, 1998) affect the simulated FLR. Lazár et al. (1997), Tomek et al. (2001), and Sušila et al. (2004) used a model similar to the one of Strasser and coworkers. In the three studies mentioned, this model was extended by a more detailed description of the exchange of $Q_B^-(H^+)_2$ for an oxidized PQ molecule from the pool; further, the effect of the rates of the S-state transitions of the OEC on the rate of $Q_A$ reduction was implicitly taken into account. This extended model was subsequently used for a detailed analysis of the effect of PS II herbicides on the FLR in leaves (Lazár et al., 1997), the dependence of the shape of the FLR on intensity of exciting light in leaves (Tomek et al., 2001), and the effect of the light gradient within thylakoid membrane samples on the shape of the FLR (Sušila et al., 2004).

We note that all studies mentioned above, with the exception of the work by Strasser and Stirbet (1998), assumed the presence of an unknown component X, which accepts electrons from
Q_B with a high rate. Without this assumption, no typical O–J–I–P FLR could be simulated (the I step would appear below 10 ms and it would be too high; data not shown in these studies).

3. Extended Two-electron Gate Models

The TEG model was further extended to include a more detailed description of the electron transport reactions occurring at the donor and the acceptor sides of PS II reaction centers. These extended TEG models were used for modeling of the O–J–I–P FLR: Stirbet et al. (1998) extended the TEG model, including the S-state transitions of the OEC. However, simulations based on this model rise too fast, showing particular steps of the FLR at times considerably shorter than the experimentally observed times. This model was subsequently used to fit the experimental FLR (Stirbet and Strasser, 2001). The fits confirmed the problems with the earlier simulations: to obtain a good fit, the model electron transport reactions on the acceptor side of PS II had to be much slower than observed experimentally. Even consideration of Q_B non-reducing centers and the exponential law for the exchange of a molecule Q_B^- (H^+)_2 with a PQ molecule from the pool – similarly to the one used by Renger and Schulze (1985) – did not lead to a better result (Stirbet et al., 2001). In follow-up studies, the TEG model was adapted considering the role of Pheo (Strasser and Stirbet, 2001) and Pheo and P680 (Stirbet and Strasser, 2001). The goal of these studies was to explore and discuss alternative definitions of variable Chl a fluorescence and closed RCs (see Section II.F for a description of these different definitions). But for all the explored definitions the fitted values of some of the model parameters (rate constants) were far from the values found in the literature.

Although the basis of the TST model was originally formulated considering redox states of only P680, Pheo and Q_A (Vredenberg, 2000; see Section II.F), the TST model was later extended by inclusion of Q_B, Y_Z and S-states of OEC (Vredenberg et al., 2001). The extended TST model can therefore be considered as an extended TEG model. A special feature of the extended TST model is that it assumes that Y_Z^- is a quencher of fluorescence (Vredenberg et al., 2001; Vredenberg, 2004, 2008a, b). The extended TST model is able to simulate a FLR curve, which reaches its maximum at the position of the I step and only one step (I step) appears between the F_0 and F_M levels. But when the I–P phase is ascribed to the effect of ΔΨ and implicitly included in the model (Vredenberg et al., 2006), a typical O–J–I–P FLR can be obtained. For more details on Vredenberg’s models see Chapter 6.

4. Combined Reversible Radical Pair and Two-electron Gate Models

Particular models of PS II, consisting of a combination of the RRP and TEG models are described below and the energy/electron carriers and processes considered in each particular model are summarized in Table 5.3 to allow an easier comparison of the various approaches. Baake and Schlöder (1992) used a TEG model in combination with a quasi-steady-state solution of the RRP model to fit FLRs measured at three low intensities of exciting light (O–I–P transient). Despite the fact that they obtained a good fit, a systematic deviation between theory and experiments was detected around the position of the I step. Therefore, Baake and Schlöder (1992) extended their model to take into account the Q_B non-reducing RC II s or electron outflow from reduced PQ to PS I and Fd, but none of these extensions led to a better fit.

The models of Andrei Rubin and coworkers (Riznichenko et al., 1999, 2000; Lebedeva et al., 2000, 2002; Belyaeva et al., 2006; see Chapter 7 by Andrew Rubin and Galina Riznichenko in this book) differ from the other models, because more emphasis is placed on processes that are only indirectly related to electron transport. In all these studies, the pH of stroma and lumen affected the rates of Q_B^- protonation and Q_B^- /PQ exchange (see Section II.E) and rate of P680^+ reduction, respectively. Further, in all the studies mentioned above, ΔΨ affected rates of electron transport reactions directed perpendicularly to membrane surface. A general concept of description of electron transport through all carriers and protein complexes in thylakoid membrane was introduced by Riznichenko et al. (1999, 2000) and later elaborated by Lebedeva et al. (2002) (see Section III.B.5), but Lebedeva et al. (2000) and Belyaeva et al. (2006) used a PS II model
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Table 5.3. Summary of energy/electron carriers and processes considered in the PS II models, consisting of a combination of the RRP and TEG models, used for simulations of the FLR. The carriers are as follows: Chl, Chl, and Chl, – all chlorophylls of whole, peripheral and core, respectively, LHC II; S-states – the S-states of OEC; Y, – tyrosine; Z, P680 – a PS II electron donor (chlorophyll); Pheo – the primary PS II electron acceptor (pheophytin); QA – the primary quinone PS II electron acceptor; QB – the secondary quinone PS II electron acceptor; PQ – plastoquinone. Energy/electron carriers connected by a hyphen were considered to be in kinetic equilibrium (for more details, see the text and the particular studies mentioned).

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Models of PS II considered in all the above research, originating in the group of Andrei Rubin, consist of a combination of RRP and TEG models, further treating the PS II model in different ways. Based on time hierarchy of energy and electron transport reactions occurring in PS II, a model consisting of 28 redox states of PS II, was reduced (see Section II.G) by Lebedeva et al. (2000) to a PS II model consisting of ten redox states. Time courses of the ten redox states of PS II were calculated first. On the basis of these ten time courses the time courses of all the 28 redox states of PS II were calculated using a set of equations “connecting” the redox states of the reduced PS II model with the redox states of the original PS II model. The model used by Lebedeva et al. (2000) considered only H ions, whose concentrations in stroma and lumen were constant in time. As noted by Lebedeva et al. (2000), the constancy of ΔΨ and of concentrations of H ions used in their model are reasons why fluorescence only monotonously increases and no typical O–I–D–P/O–J–I–P transients were simulated. We note that the model reduction used by Lebedeva et al. (2000) might as well be a reason for the unsuccessful simulation, because the model reduction procedure always causes a loss of information.

Belyaeva et al. (2006) considered the PS II model (with 28 redox states) mentioned above, with time-dependent concentrations of oxidized and reduced PQ, but they did not apply any reduction of the model. The original PS II model did not consider YZ (an intermediate between Mn complex and P680) and cycling of S-states and described reduction of P680 by a single rate constant. Also, fluorescence quenching by P680 and oxidized PQ pool was not considered. On the other hand, because the main goal of Belyaeva et al. (2006) was to simulate the fluorescence sig-
nal after the application of a strong and short laser flash (see Steffen et al., 2005), other processes were included in this model: nonradiative charge recombination between $P680^+$ and Pheo$^-$ as well as between $Q_Y^-$ and a positive charge stored on the donor side of PS II. With respect to the considered ions and $\Delta \Psi$, the same “constraints” were used by Belyaeva et al. (2006) as mentioned above for the paper by Lebedeva et al. (2000). The O–J–I–P-transients, simulated on the basis of this model (Belyaeva et al., 2006), were characterized by an O–J rise that was dominant (on the order of 90% of the total FLR) and the J and I steps occurred sooner than observed experimentally (e.g., Strasser et al., 1995; Fig. 5.1A); J was below 1 ms and I after a few ms. The dominance of the O–J rise could be due to the use of a high value for the rate constant of ES formation ($k_L = 6.000 \text{s}^{-1}$) and also to the fact that quenching by neither $P680^+$ nor PQ pool was considered. As the shape of the fluorescence transient simulated in Belyaeva et al. (2006) resembles the shape of the transients simulated in Lebedeva et al. (2000), the time independence of $\Delta \Psi$ and of amount of $H^+$ ions assumed in Belyaeva et al. (2006) may also be the reason for the dominant J step in the simulations presented by Belyaeva et al. (2006).

Lazár (2003) and Zhu et al. (2005) used very similar models. In both cases, a combination of TEG, RRP and Kok models was used to model the FLR measured upon high intensity of exciting light (O–J–I–P transient). In both models, the existence of quenching by $P680^+$ (Butler, 1972; Bruce et al., 1997) and PQ (Vernotte et al., 1979; Kurreck et al., 2000) was assumed. There are, however, some differences. Lazár (2003) takes the S-state dependence of the reduction of $P680^+$ by $Y_Z$ into account, whereas Zhu et al. (2005) use a single rate constant, but they split the light harvesting antenna of PS II (LHC II) into its peripheral and core parts – a detail that is missing in the model of Lazár (2003). With respect to the model of Xinguang Zhu and his coworkers, Laisk et al. (2006) remarked that this model had ignored that the reactions within PS II electron transport should have been restricted to acceptors and donors within the same complex (i.e. first order kinetics should have been used instead of second order kinetics; see Section II.E for a discussion of this point). Lazár (2003) had also used second order kinetics for a description of some reactions (on the PS II donor side) but in an improved web-based version of this model (Lazár, 2005) it was modified. Belyaeva et al. (2006) wrote that the description of the exchange of $PQH_2$ at the $Q_B$ site in the model of Lazár (2003) is insufficient in the sense that it does not take into account changes in the stromal pH (see Section II.E). The model of Lazár (2003) has now been modified in several respects (Jablonsky and Lazar, 2008), the main modification being the introduction of the so-called intermediate S-states. This enabled the simulation of oscillations in oxygen evolution and fluorescence signal under a train of single-turnover flashes without consideration of the miss and double-hit parameters defined by Kok et al. (1970). This modification however only leads to a slightly higher and less pronounced J step in the FLR (data not shown in that study). As a consequence the shape of the FLR remains qualitatively unchanged compared to the FLRs simulated on the basis of the original model of Lazár (2003).

Both the simulations of Lazár (2003) and Zhu et al. (2005) reach a maximum after 30–40 ms. However, the interpretation of this level is quite different. Lazár (2003) concluded that it was not possible to simulate the I–P phase with his own model, whereas Zhu et al. (2005) concluded that their 40 ms point represented P. Looking at the kinetics of transients simulated by Zhu et al. (2005), two steps between the $F_0$ and $F_M$ levels are observed. However, the steps occur sooner (I at about 600 $\mu$s, I at about 3 ms and P at about 40 ms) than observed experimentally (see e.g., Strasser et al., 1995; Fig. 5.1A). The incorrect time dependence may, however, also be due to an incorrect assignment of the phases. The different steps would come very close to an O–K–J–I transient. An argument that can be made against this reasoning is that according to the model of Zhu et al. (2005), all $Q_A$ was reduced after 40 ms and therefore it was by definition P. See discussion below for this point. Laisk et al. (2006) criticized the Zhu et al. (2005) model also for ignoring the kinetic role of PS I. In the Laisk et al. (2006) model (see below) the I–P phase is assigned to the slow-down of electron transport due to the reduction of PS I acceptors. A further comparison between models of Lazár (2003) and Zhu et al. (2005) shows that there is an important difference in the behavior of $P680^+$...
5 Modeling Fluorescence Transients

as a function of the initial S-state distribution. In the model of Zhu et al. (2005), P680\(^+\) quenching has no effect on the FLR if the starting condition consisted of mostly \(S_1\) (\(S_1/S_0 = 0.8/0.2\)), but a huge effect was observed if there was initially mostly \(S_0\) (\(S_1/S_0 = 0.1/0.9\)). The \(S_0\)-effect was due to the choice of \(k_{o1}\) (rate constant for the \(S_0\) to \(S_1\) transition) of 50 s\(^{-1}\) compared to 20,000 s\(^{-1}\) in the model of Lazár (2003) (see Razeghifard et al., 1997 for a discussion of these values). Farred light is a very inefficient excitation source for PS II (Pettai et al., 2005; Schansker and Strasser, 2005). As argued by Schansker and Strasser (2005) this means that leaves that were pre-illuminated with FR light for 10 s will start out with a mixture of S-states. In such samples, the O–J rise is much slower in a way that is quite similar to transients simulated by the model of Lazár (2003) for high initial concentrations of \(S_2\) and \(S_1\) (cf. Schansker and Strasser, 2005, and Fig. 5.6 in Lazár, 2003). If the J step of Zhu et al. (2005) would be \(K\), then the break in the kinetics of the transient at approximately 300\(\mu\)s simulated for the case that there is 100% \(Q_B^\text{F}\) in the starting state (trace 0:1 in Fig. 5.5d of Zhu et al., 2005) does not agree with what is observed experimentally, since experimental curves of control samples (no treatments) have quite smooth kinetics between 20\(\mu\)s and 2 ms (see curves a and c in Fig. 5.1B). However, if there is no or very little \(Q_B^\text{F}\) initially, the break disappears and the O–J I rise of Zhu et al. (2005; Fig. 5.5d, trace 1:0 therein) starts to look like the O–J rise observed experimentally. In this respect it may be noted that the study of Schansker and Strasser (2005) implies that in well dark-adapted leaves the initially \(Q_B^\text{F}\) concentration is low.

The studies discussed above show that it is difficult to simulate a complete O–J–I–P transient on the basis of models which solely describe PS II reactions. Lazár (2003) concluded that he was only able to simulate the O–J–I transient on that basis. And the same was probably true for Zhu et al. (2005). As noted above, even for the simulation of the O–J–I transient it is necessary to assume that there is quenching by P680\(^+\) (Butler, 1972; Bruce et al., 1997) and PQ (Vernotte et al, 1979; Kurreck et al., 2000). But as shown experimentally by Tóth et al. (2005), quenching by PQ probably does not occur in leaves, though it can be observed in thylakoid preparations of different integrity. The inability to simulate the whole transient does not seem to be due to a lack of understanding of the kinetics of the system. As discussed above, the slow-down of the O–J rise modeled by Lazár (2003) agrees quite well with the O–J rise following a FR pre-illumination that creates similar conditions. One solution to this problem is to introduce some tricky assumptions. As noted in Section III.B.2, in several older models a fast outflow of electrons from \(Q_B^\text{T}\) towards a component X was introduced in order to slow down the FLR. Lazár (2003) demonstrated that by introducing slowly reducing PQ pool, and special (slow) properties of PS II, \(Q_B^\text{T}\) non-reducing centers, simulated FLR transients approach the experimentally measured transients. However, the dark-adaptation kinetics of the O–J–I–P transient (Schansker et al., 2005) provides very little support for such innovations. Another approach would be to admit that the redox state of \(Q_A\) is perhaps the dominant but not the only important determinant of the fluorescence yield and that the type of heterogeneity introduced by Delosme (1967) – whatever its underlying mechanism – provides us with the tool to simulate the whole O–J–I–P transient. Certain experimental observations (see Section II.F), as well as the above-discussed simulations, point in this same direction.

5. Complex Models

The results described in the previous sections indicate that even very complicated models describing the reactions occurring in PS II are not able to simulate the correct O–J–I–P FLR curve. It shows that consideration of only the reactions occurring in PS II is not enough, and that more reactions in the electron transport chain must be considered. Several complex models are described below and the components and processes considered in each particular model are summarized in Table 5.4 for easier comparison.

Riznichenko et al. (1999, 2000) considered PS II and the following electron transport carriers/systems: PQ, cyt b\(_6\)f, PC, PS I, and Fd in their FLR model. They also considered cyclic electron transport around PS I (from Fd to cyt b\(_6\)f), formation of the transmembrane proton gradient, \(\Delta\mu\text{H}^+\), and its role in ATP synthesis, passive leakage of H\(^+\), K\(^+\), and Cl\(^-\) ions across
Table 5.4. Summary of model components and processes considered in the complex models used for simulations of the FLR. Particular energy/electron carriers (in parentheses) used for a description of the given electron transport system are as follows: Chl – all chlorophylls of whole LHC II; OEC – oxygen evolving complex; S-states – the S-states of OEC; P680 – PS II electron donor (chlorophyll); Pheo – primary PS II electron acceptor (pheophytin); QA – primary quinone PS II electron acceptor; Qb – secondary quinone PS II electron acceptor; PQ – plastoquinone; FeS$_{cyt}$ – iron–sulfur center of cyt b$_f$; f – cytochrome f; b$_L$ – low potential form of cyt b; b$_H$ – high potential form of cyt b; Q$_O$ – luminal site of cyt b$_f$ where plastoquinol is oxidized; Q$_I$ – stromal site of cyt b$_f$ where plastoquinone is reduced; P700 – PS I electron donor (chlorophyll); A$_0$ – primary PS I electron acceptor (chlorophyll); A$_1$ – secondary PS I electron acceptor (phyloquinone); FX – PS I electron acceptor (iron–sulfur center); Fd – ferredoxin; NADPH – reduced nicotinamide adenine dinucleotide phosphate. Energy/electron carriers connected by a hyphen were considered to be in kinetic equilibrium (for more details, see the text and the particular studies mentioned).

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<tr>
<td>Model components</td>
<td>Reduced PS II model (Chl-P680, Pheo, QA, Qb)</td>
<td>PS II (Chl-P680, Pheo, QA, Qb)</td>
<td>PS II (QA, Qb)</td>
<td>PS II (OEC-Chl-PS II)</td>
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<tr>
<td></td>
<td>PQ pool</td>
<td>PQ pool</td>
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<td></td>
<td>Cyt b$<em>f$ (FeS$</em>{cyt}$-f, b$_L$, b$_H$)</td>
<td>Cyt b$<em>f$ (FeS$</em>{cyt}$-f, b$_L$, b$_H$)</td>
<td>Cyt b$_f$ (cyt b$_f$-PC)</td>
<td>Cyt b$_f$ (f, b$_L$, b$_H$)</td>
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<tr>
<td></td>
<td>PC</td>
<td>PC</td>
<td>PC (PC-P700)</td>
<td>PC</td>
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<td></td>
<td>PS I (P700, FePS$_1$)</td>
<td>PS I (P700, FePS$_1$)</td>
<td>PS I (PC-P700)</td>
<td>PS I (P700, FX$_X$)</td>
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<tr>
<td>PS I cyclic electron transport</td>
<td>Fd, NADPH</td>
<td>Fd</td>
<td>Fd–NADPH</td>
<td>Fd, NADPH</td>
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<td>Fluorescence during FLR</td>
<td>Deactivation of ES</td>
<td>Deactivation of ES</td>
<td>Proportional to amount of Q$_{QA}$</td>
<td>Proportional to amount of reduced PS II</td>
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<td>Other processes that were</td>
<td>Concentrations of H$^+$, K$^+$ and Cl$^-$ in stroma and lumen are variable and pH of stroma and lumen affects rates of protonation of Q$_{QA}$ and Q$_B$ / PQ exchange and of P680$^+$ reduction, respectively; transmembrane electric potential is variable in time and affects rates of reactions directed perpendicularly to membrane surface</td>
<td>–</td>
<td>Reactions occurring in the stroma (Calvin–Benson cycle and starch synthesis) and in the cytosol (sucrose synthesis)</td>
<td>–</td>
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5 Modeling Fluorescence Transients

the membrane, and the dependence of rate constants on stromal and luminal pH and on $\Delta \Psi$ (see Section III.B.4 and Chapter 7). Their original PS II model consisting of 21 redox states (those with vacant $Q_b$ pocket were not considered, in comparison with the PS II model with 28 redox states mentioned above) was reduced in Riznichenko et al. (1999, 2000) to one consisting of only four redox states (Lebedeva et al., 2000, see Section III.B.4). In the model of Galina Riznichenko, Andrei Rubin and coworkers, it is assumed that PQ and PC in dark-adapted samples are in the oxidized state. In our opinion, these assumptions are incorrect. In general, PC is considered to be reduced after dark adaptation (see e.g. Oja et al., 2003; Schansker et al., 2003) and the O–I–D–P transients are characteristic of samples with an initially partially reduced PQ pool (e.g. Kautsky et al., 1960). Riznichenko et al. (1999) assumed that the O–I rise is due to a $\Delta \Psi$-induced slow-down of several electron transport reactions (appearance of the I step corresponds with the maximum rate of $\Delta \Psi$ increase) and that the subsequent I–D decline is due to a decline in the rate of $\Delta \Psi$ increase due to $K^+$ and $Cl^-$ fluxes. The following D–P FR (see electron back-pressure caused by the accumulation of electrons on the PS II acceptor side.

Lebedeva et al. (2002) considered PS II and the following electron transport reactions, as well as all other properties of the model of Riznichenko et al. (1999, 2000), except that all the 28 redox states (without any reduction) were used by Lebedeva et al. (2002). A typical feature of the transient modeled by Lebedeva et al. (2002) is the slow rise of the I–P phase. In leaves, the I–P rise is much steeper because ferredoxin-NADP$^+$-reductase (FNR) is inactive in darkness, transiently blocking electron transport beyond Fd (Satoh and Katoh, 1980; Carrillo and Vallejos, 1987; Foyer et al., 1992). If this had been taken into account, a much better correspondence between model and experiment could have been obtained (see next section). By ignoring inactive FNR, the Lebedeva et al. (2002) model had much more electron transport and, therefore, a much greater release of protons into the lumen during the I–P phase than would occur under in vivo conditions. However, this effect may be canceled in part if the activation of ATP synthase was slower than instantaneous as assumed in that study. A curiosity of the form of the simulated transient is that it looks as if it was pre-illuminated with FR (cf. Schansker and Strasser, 2005). Thus, even this complex model was not able to simulate the positions and heights of the particular steps of the experimental O–J–I–P FR.

A model including PS II, PQ, cyt b$_6$f, PC, PS I, Fd and cyclic electron flow, but not the processes on the donor side of PS II, was used by Kroon and Thoms (2006) for phytoplankton photosynthesis. It used second order instead of first order kinetics (see Section II.E) for some reactions occurring in PS I. For simulation of the FLR, the stoichiometry between the components of the photosynthetic electron transport chain was considered to be $C_{PS II}:C_{b_6f}:C_{PS I}:C_{PQ}:C_{PC}:C_{Fd} = 1:1:1:4:1:1$. We note that in higher plants there is more than one molecule of PC and Fd per PS I (Terashima and Inoue, 1985; Haehnel et al., 1990; Klughammer and Schreiber, 1991; Holtgreve et al., 2003) and this may also be the case for phytoplankton. Kroon and Thoms (2006) simulated the FLR measured using the FRR technique (see Section I.B). Quite a few differences were observed comparing the simulations with the experimental transients of Kolber et al. (1998). A problem with the transients of Kolber et al. (1998) is that for the highest excitation pressure (4,000 quanta $RC^{-1} s^{-1}$) incomplete O–J–I–P transients were obtained (only O–J–I). Assuming that the simulated transients were complete, they had either a very broad J-step and no I–P phase or no J–I rise and an I–P phase that rose too fast. Taking into account that the number of electron acceptors that Kroon and Thoms (2006) assumed to exist beyond the cyt b$_6$f complex is too low, a fast I–P rise may not be an unexpected result. The re-oxidation of the electron transport chain following P (decline of the fluorescence intensity) for the lower light intensities is also far too limited compared to the curves measured by Kolber et al. (1998). The calculated cyclic electron transport rates around PS I as a function of the light intensity seem to underestimate the influence of the strong reduction of the available substrate (oxidized PQ) at the higher light intensities.

The most complex model (as for the number of considered reactions) employed so far for the FLR was used by Laisk et al. (2006; see also Section IV.A). Their model consists not only of
the light reactions occurring in thylakoid membranes but also of reactions occurring in the stroma and the cytosol (see Chapter 13 by Agu Laisk, Hillar Eichelmann and Vello Oja in this book). Laisk et al. (2006) used a rather simplified description of the light reactions, nevertheless, cyclic electron transport around PS I (from NADPH to the PQ pool) was considered. In the model, many electron-transport components were considered to be in a kinetic equilibrium (see Table 5.4) and redox state of PS II was described only by one state variable, which could be reduced or oxidized. On the other hand, Calvin–Benson cycle and starch synthesis occurring in the stroma, as well as sucrose synthesis occurring in the cytosol, were described in detail, including the activation of several enzymes that are under the control of the thioredoxin system. Reducing the acceptor side of PS II to a single state variable possibly explains why Laisk et al. (2006) were able to simulate the whole electron transport chain with their model. However, due to the fact that they ignored the presence of inactive FNR, the I–P rise is much slower than in comparable experimental transients. Another discrepancy between their model and the experimental transient is that the I step in the model is reached after almost 200 ms, whereas in experimental transients it is almost independent of the intensity of excitation light (cf. e.g. Tomek et al., 2001; Schansker et al., 2005). Lowering the light intensity lowers the relative amplitude of the I step but it is still observed around 30 ms. On the other hand, as also noted by Laisk et al. (2006), the rate of CO₂ uptake increases much faster in the model than in the experimental measurements. As will be discussed in the next section this may also be related to the absence of inactive FNR in the model of Laisk et al. (2006). With respect to the I₈₂₀ signal (the authors measured it at 810 nm), Laisk et al. (2006) model the FR-induced oxidation of the electron transport chain, and the dark recovery kinetics of the I₈₂₀ signal of samples darkened after reaching steady-state conditions. However, the I₈₂₀ kinetics paralleling the FLR was not modeled. As noted above there are major discrepancies between model and experiment and it remains to be seen if e.g. the introduction of inactive FNR in the model will produce a better simulation.

To test whether reactions occurring in and around PS I affect the FLR as suggested before (Kautsky et al., 1960; Munday and Govindjee, 1969; Satoh and Katoh, 1981; Hansen et al., 1991; Schansker et al., 2003, 2005), D. Lazár (2007, unpublished) used a model which consisted of PS II (considering S-states, P₆₈₀, Qₓ, and Q₉₆), PQ pool, cyt b₅₆, PC, PS I (considering P₇₀₀ and Fₓ), Fd and FNR, where Fd dependent cyclic electron transport around PS I was also considered. The goal was to simulate the FLR and light-induced changes in the I₈₂₀ signal paralleling the FLR. Results of simulations of the FLR and I₈₂₀ signal, as well as time courses of state variables responsible for these quantities, are shown in Fig. 5.6A. Although the
modeled FLR better simulates the one measured with thylakoid membranes than that with leaves (Fig. 5.1A), the model was also able to qualitatively simulate changes in the FLR and I$_{820}$ signal (data not shown) as measured with different intensities of exciting light (Ilík et al., 2006) as well as that caused by the application of DBMIB and 1, 1’-dimethyl-4, 4’-bipyridinium-dichloride (methylviologen, MV) (c.f. Fig. 5.6B and Schansker et al., 2005).

The results described in this section show that even if very complex models are used for simulations of the FLRs, it is still difficult to get a perfect match with experimentally obtained transients. We also note that the description of PS II gets worse as the models become more complex (e.g. by introducing a single state variable to describe the acceptor side of PS II, which can be oxidized or reduced as Laisk et al. (2006) did in their model; see Table 5.4). This can also be the reason why some of these complex models were not very successful in simulating the form of the FLR.

IV. Modeling the Whole Fluorescence Induction

Few models simulate the FLD after the P step. Therefore, a discussion of processes which could be important for simulation of the whole FLI is provided in this section together with a description of models simulating fluorescence oscillations which sometimes occur in the FLI.

A. Experimental Observations and Models

As mentioned in Section I.2, the FLI consists both of a fast FLR and a subsequent slower FLD. So far, only modeling of the FLR was discussed. In the older literature a considerable effort was devoted to the characterization of the FLD (reviewed by Papageorgiou, 1975; Briantais et al., 1986; Govindjee and Satoh, 1986). However, a full experimental description of processes involved was never achieved and the studies of the processes occurring on this time scale took a completely different direction with the introduction of the saturating pulse method (reviewed by Roháček 2002; Schreiber, 2004; Baker, 2008). On the other hand there exist very successful models of the Calvin–Benson cycle reactions (Farquhar et al., 1980; Von Caemmerer, 2000; Bernacchi et al., 2001; Ethier and Livingston, 2004; Chapter 9 by Susanne von Caemmerer, Graham D. Farquhar and Joseph Berry and Chapter 10 by Carl J. Bernacchi, David Rosenthal, Carlos Pimentel, Stephen P. Long and Graham D. Farquhar in this book).

For a model of the FLD it is very important to define the starting point, in other words, what is the state of the photosynthetic apparatus at the P step. Kautsky et al. (1960) were probably the first to describe a transient block of electron transport in dark-adapted samples. This observation was confirmed by Munday and Govindjee (1969) and about 10 years later Satoh and Katoh (1980) and Satoh (1981, 1982) localized this block between Fd and NADP$^+$ (c.f. Fig. 5.6B and Schansker et al., 2005). The properties of FNR were studied in more details by N. Carrillo and coworkers (reviewed by Carrillo and Vallesjos, 1987). The presence of this transient block of electron transport also plays an important role in the studies of the O–J–I–P-transient of Schansker et al. (2005, 2006). Despite extensive experimental support for the presence of inactive FNR in dark-adapted leaves and algae, it is often neglected. Both the model studies of Lebedeva et al. (2002) and Laisk et al. (2006) (see Section III.B.5 and Chapter 13) assume that there is a free outflow of electrons at the acceptor side of PS I. This has of course a quite drastic effect on the validity of the obtained simulations. In their models many more electrons flow through the electron transport chain and many more protons are transferred from stroma to lumen by the time the P step is reached. For inactive FNR, the number of electrons that have to flow through the electron transport chain (essentially to reduce $Q_A + PQ + Fd$) to reach P step can be estimated and that would also allow an estimate of the number of protons that are transferred to the lumen during this time interval. However, to determine the built-up of a proton gradient, the activation time of ATP synthase also has to be known. The activation kinetics of ATP synthase is quite complex (Groth and Strotmann, 1999; He et al., 2000). An electrochemical proton gradient is needed to activate ATP synthase, but the necessary gradient is smaller once a sulfur bridge in the protein is reduced by thioredoxin. Therefore, we raise the
There is much more heterogeneity in the kinetics of the FLD between species (see Papageorgiou et al., 2007) and an important factor for this is the time needed to activate FNR. FNR is activated in a few hundreds of milliseconds in Trebouxia possessing lichens (Ilík et al., 2006), Ginkgo biloba (G. Schansker, 2005, 2008, unpublished) and many conifers (Schansker et al., 2008). In angiosperms, however, the activation of FNR is quite slow. The secondary fluorescence kinetics observed in lichens, Ginkgo biloba and conifers is completely missing in angiosperms. This phenomenon may explain the differences in complexity of the various measured FLI transients (cf. Papageorgiou and Govindjee, 1968 with Yamagishi et al., 1978). In an angiosperm like pea, swings in the fluorescence intensity beyond the P step can be created by a partial activation of FNR induced by a short pre-illumination (G. Schansker, 2002, unpublished). Accompanying swings in the $I_{620}$ signal seem to point to additional transient “traffic jams” in response to reduction of the NADP$^+$ and perhaps thioredoxin pools before the Calvin–Benson cycle is activated. From a simulation point of view the challenge will be to get good experimental data on the various processes involved.

**Limitation on the acceptor side of PS I (activation state of FNR).** As noted above, the activation of FNR in angiosperms is rather slow. Foyer et al. (1992) observed for pea plants that the NADPH/NADP$^+$-ratio did not change much during the first 10 s of illumination (750 μmol photons m$^{-2}$ s$^{-1}$) indicating that FNR was essentially inactive during this period of time. This phenomenon is also observed in the fluorescence and $I_{620}$ measurements where both signals remain high during the first few seconds of illumination. For a simulation, this means that there is nearly no electron transport during this time interval. This is something that none of the existing models take into account. Schansker et al. (2006) demonstrated that the inactivation of FNR in pea leaves is observed as the recovery of the I–P phase. Probing pea leaves 200 s after pre-illumination by red light (350 μmol photons m$^{-2}$ s$^{-1}$), it was observed that pre-illumination of at least 200 s are needed to obtain a maximum suppression of the I–P phase and therefore a maximum activation of FNR (Schansker et al., 2008).

**Transient reduction of the pool of NADP$^+$ (and thioredoxin).** As noted above, Foyer et al. (1992) observed that the NADPH/NADP$^+$-ratio during a dark-to-light transition did not change during the first 10 s. Subsequently, NADPH-levels were high between approximately 20 and 100 s of illumination. A limited availability of NADP as substrate for FNR will prolong the limitation on the outflow of electrons on the acceptor side of PS I and thereby electron flow through the photosynthetic electron transport chain. Partially pre-activating FNR by short pre-illuminations allows the visualization of small swings in the fluorescence signal and quite large swings in the $I_{620}$ signal (G. Schansker, 2002, unpublished) probably related to changes in substrate availability. The absence of such swings in well dark-adapted angiosperms should simplify the task of the modeller considerably.

**Cyclic electron transport around PS I.** Under certain conditions, a swing in the fluorescence intensity beyond the P step can be observed that is not accompanied by a change in the $I_{620}$ signal. An example of this can be found in Ilík et al. (2006) for a *Trebouxia*-possessing lichen and also in *Pinus halepensis* (Schansker et al., 2008). Ilík et al. (2006) did not observe an effect of the addition of inhibitors of cyclic electron transport around PS I on this fluorescence property. However, cyclic electron transport around PS I remains a good explanation for this phenomenon in the absence of more conclusive experimental evidence. For cyclic electron transport around PS I to occur a (partial) re-oxygenation of the PQ pool is necessary and this condition is only met following a considerable activation of FNR. This means again that this process may be of limited relevance to the kinetic changes in the FLD of angiosperm plants.

**Energy dependent non-photochemical fluorescence quenching (qE).** In Fig. 5.7 FLR-transients are shown that have been measured 10 s after a 10–600 s long pre-illumination by red light (350 μmol photons m$^{-2}$ s$^{-1}$). The high values of J steps demonstrate that the PQ pool was highly reduced under these measuring conditions and represent therefore a good alternative measure of the maximum fluorescence intensity. The figure also shows small swings in the transients in the
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Fig. 5.7. Measured Chl a FLIs (Panel A) and related changes of fluorescence at the J (the maximum between 2 and 10 ms) and P steps (the maximum between 100 and 400 ms) (Panel B). Chl a FLI was measured by PEA Senior fluorometer upon illumination with 1,800 µmol photons m$^{-2}$ s$^{-1}$ of red light with pea leaves 10 s after 10–600 s pre-illumination by red light (350 µmol photons m$^{-2}$ s$^{-1}$). Curves in panel A are shown on a logarithmic time-axis, pre-illumination times are indicated.

As noted above, qE has a major impact on the fluorescence emission of closed RCs. It is less clear if the qE reduces the excitation pressure of open RCs and if this leads to a qE-dependent reduction of linear electron transport. Schansker et al. (2006) observed that in pea leaves even at relatively high intensities of excitation light (400–500 µmol photons m$^{-2}$ s$^{-1}$) the PQ pool remains quite oxidized. Further, G. Schansker (2006, unpublished) observed that in the STN7 mutant of Arabidopsis thaliana, the absence of the kinase responsible for the phosphorylation of LHC II only causes a slightly more reduced PQ pool at lower intensities of excitation light (about 70 µmol photons m$^{-2}$ s$^{-1}$) but not at higher intensities (300–400 µmol photons m$^{-2}$ s$^{-1}$). It is another indication that qE may reduce the electron transport rate. However, this is a point that deserves further experimentation.

State I to state II transitions. Phosphorylation of LHC II may affect the fluorescence transient in several ways. A reduction of the antenna size reduces not only the excitation cross-section, it also reduces the maximum fluorescence intensity. However, the effect of state transitions on the electron transport rate is not so well defined. It is thought that, in higher plants, only a part of the RC IIs is affected by state transitions (Vallon et al., 1991; Bellafiore et al., 2005) – possibly mainly those near the margins of the grana stacks. In these regions state transitions may have, as an

presence of a more or less activated FNR. In panel B of Fig. 5.7 fluorescence at the J and P steps are plotted as a function of the length of the pre-illumination. Both parameters change in parallel indicating that this change is dominated by changes in qE. The data can be described quite well by a single exponential with a time constant of approximately 90 s. During the first 10 s of illumination, no effect on the $F_P$-value was observed, which agrees with the slow activation of FNR noted above, but raises questions with respect to the role of the lumenal pH during this part of the FLI. The development of qE is closely related to the lumenal pH, which in turn depends both on the activity of ATP synthase and the electron transport activity. A description of the development of qE will therefore also take care of the pH-related changes in the lumen. A considerably more sophisticated approach to the development of qE during the P to S decline would be to study changes in the fluorescence lifetime. An example of such a study on the alga Chlamydomonas reinhardtii can be found in Holub et al. (2007). The fact that the development of qE can be described by a single exponential indicates that it may be relatively simple to simulate this parameter. Using mutants, whose development of qE is slowed down (e.g. the one described by Govindjee and Spilotro, 2002), it is possible to obtain modified FLDs.

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additional effect, that they reduce the extent of stacking (Vallon et al., 1991; Trissl and Wilhelm, 1993; Pesaresi et al., 2002). This may increase the extent of spillover between PS II and PS I antennae. Further, it has been shown that the kinase that phosphorylates LHC II is inhibited at high light (Rintamäki et al., 1997). At the same time it is possible that qE dominates anyway at higher light intensities. As noted above, in the kinase-less mutant of Arabidopsis thaliana an effect of this mutation on the redox state of the PQ pool is only observed at lower light intensities. All these complications could mean that for a given plant species, the light intensity dependence of state transitions has to be determined to determine the influence of this process on the FLD.

An additional problem in this respect is due to the absence of clear fluorescence parameters that can help to define the development of a state transition during photosynthetic induction. Schreiber et al. (1995) have suggested for Chlamydomonas reinhardtii and Synechocystis PCC 6803 that a reduction of the J-I amplitude could be an indicator for state transitions. However, proof for this suggestion in higher plants is at the moment missing. Nevertheless, a model which includes an effect of the state transitions was constructed and used for simulation of the FLI by Gordienko and Karavaev (2003). These authors calculated response curves for P700 on switching between FR and white light. Following the switch the calculated curves pass through a minimum before reaching a steady-state level. The steady-state level in the model of Gordienko and Karavaev (2003) depends also on the parameter related to the LHC II kinase, which leads to a redistribution of excitation light between PS II and PS I. In addition, the calculations by the authors show that, in their model, changes in the phosphorylation level of LHC II (leading to changes in the light distribution between both photosystems) have a strong effect on the fluorescence kinetics beyond the P level.

As noted in Section II.B, it makes sense for the simulation of the O-J-I-P FLR to work with transients induced by high light intensities. However, for the kinetics beyond the P step – that is dominated by biochemical processes – such an approach makes less sense. As discussed above, the P-level is a well-defined situation due to the presence of inactive FNR. In this respect, a light intensity that is just saturating for the P step would probably create a very similar starting state for the FLD compared to the one obtained with high light intensities. Given the limited kinetic information that can be derived from an angiosperm FLD, an alternative approach would be to simulate probe pulses that can be given at various times following a pre-illumination representing a partial to full fluorescence transient (Fig. 5.7; Schansker et al., 2008).

B. Oscillations in the Fluorescence Intensity

As mentioned in Section I.B, upon certain conditions complex damped oscillations can be induced and measured in the FLD kinetics (Walker et al., 1983; Sivak and Walker, 1985). The oscillations can be detected not only in the fluorescence signal, but also in other parameters related to photosynthetic activity. Several hypotheses have been put forward to explain these oscillations (Lazár et al., 2005b and references therein). The oscillations in the FLD were theoretically simulated based on models, which described electron transport within thylakoid membrane, leading to the formation of ATP and its subsequent use in the Calvin–Benson cycle (Karavaev and Kukushkin, 1993; Khuznetsova and Kukushkin, 1999). A similar model, but then extended by cyclic electron transport around PS II, was published by Kukushkin (1997). Rovers and Giersch (1995) used a very simple model to describe the oscillations, describing the formation of ATP coupled to the formation of NADPH, which are both subsequently used in the Calvin–Benson cycle. The Calvin–Benson cycle in this model was described by the action of its two kinases (3-phosphoglycerate kinase and ribulose-5-phosphate kinase). A slightly extended version of the previous model was used by Lazár et al. (2005b). In this model the Calvin–Benson cycle was described in more detail by inclusion of the activation of ribulose-1,5-bisphosphate carboxylase/oxygenase by its activase.

The experimental conditions by which the oscillations are usually induced and measured (a re-illumination of the sample or a perturbation of the steady state by a change in the gas phase) differ from standard measurements of the whole FLI. Even so, the interpretation and modeling of
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the oscillations performed so far might be helpful in understanding and modeling of the whole FLI.

V. Conclusions and Future Perspectives

Despite the extensive work that has been done in the field of mathematical modeling of the FLR it has, so far, only been possible to obtain a qualitative simulation. In those cases where a quantitative agreement was achieved (by means of fitting of model parameters), the parameters had values different from the ones known from the literature or the model structure was incomplete (e.g. some PS II components and/or reactions were missing). Nevertheless, even a qualitative agreement between theory and experiment is a big step forward for a system as complex as the FLR.

How to proceed from here? We expect future progress from several approaches. In the first place, it is possible to work with a larger number of well-defined FLRs. So far, most work has concentrated on two situations: uninhibited, dark-adapted samples and DCMU-treated samples. An alternative possibility would be to simulate DBMIB-treated samples. As shown by Schansker et al. (2005), DBMIB has two major effects on the FLR: it blocks electron flow at the cyt b6f complex, reducing the transient to two steps, and DBMIB molecules affect electron flow at the Qb site. The J-level goes up as the DBMIB concentration in the thylakoid membrane increases. Another possibility would be to work with samples that are partially anaerobic. A recent study by Tóth et al. (2007b) has shown that such FLRs can be well understood and described assuming that anaerobiosis mainly affects the redox state of the PQ pool (Kautsky et al., 1960; Schreiber and Vidaver, 1974). For models including a description of the FLD, as noted above, a better definition of the P-level is needed. None of the few existing models (see Section IV.A) have taken all the experimental knowledge of the state of the photosynthetic system at P into account. As a consequence, they have overestimated both electron flow and transfer of protons into the lumen during the first second of induction.

Progress may also be expected from the further increase of our knowledge of the physiology of the chloroplast. As shown above, several heterogeneities (PS IIα/β, PQ pool size, light gradient) have been explored in simulations. However, more quantitative data, especially for the in vivo situation, on these processes, together with the effects of the compartmentalization of the various forms of the electron transport components inside the chloroplast on activity would help to restrain future models. From a modeling point of view, the application of a control analysis (Fell, 1992; Visser and Heijnen, 2002) may be useful, allowing a more precise quantification of the extent by which a reaction or mechanism affects or drives a given property of the FLR/FLI (e.g. Lazár et al., 2005a, b).

A last point would be conceptual. In the majority of cases modeling is based on the principle that the redox state of QA is the major determinant of variable fluorescence. As noted above, there are several experimental observations that are at odds with this concept. At the same time, such a model makes it also difficult to take PS I reactions into account, since the impact of the light-induced changes in the redox state of PS I on QA-redox state are much smaller than the amplitude of such changes observed experimentally. Writing these words 40 years after the introduction of the photochemical and thermal phases by Delosme (1967) it is, perhaps, time to explore different versions of models considering two determinants of the fluorescence yield more seriously.

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Materials and methods

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