Chapter 3

The D1 Protein: Past and Future Perspectives†

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Summary

The chloroplast-coded D1 protein of Photosystem II (PS II) is the major membrane protein synthesized within the plastid. It is involved in light-dependent electron transport, is a major target for photosynthesis herbicides and is universal to oxygenic phototrophs. The defining feature of D1 is its rapid turnover in spite of its being a structural component of the PS II reaction center core. Processing of nascent D1 precursor (33.5–34 kDa) occurs on unstacked stromal lamellae. The mature protein (32 kDa) then migrates to the grana where an initial scission occurs producing a 23 kDa N-terminal degradation fragment. Post-translational and reversible palmitoylation and phosphorylation accompany the protein along its life cycle. Both anabolism and catabolism of D1 are photoregulated, with synthesis coupled to phosphorylation but degradation coupled to PS II electron transport. Dephosphorylation of D1, in turn, is regulated by PS I excitation. Thus, the phosphorylation state of the protein is sensitive to the relative energy distribution between the two photosystems. Beyond redox regulation of D1 phosphorylation, an internal, circadian clock exerts overriding control. Two photosensitizers are involved in D1 degradation: chlorophyll pigments in the visible and far-red regions of the spectrum, and plastosemiquinone in the UV-B region. D1 degradation in visible light is a process only marginally overlapping with photoinhibition and overwhelmingly associated with fluences limiting for photosynthesis. Mixing physiological levels of visible and UV-B radiances leads to synergistic effects such that above a critical threshold of UV-B, the D1 as well as its sister protein, D2, both are targeted for accelerated degradation. These and other D1 protein studies, mainly carried out with intact Spirodela plants during the past 25 years in the authors' laboratories, are presented in a historical perspective.

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†This account does not intend to survey the field but rather to present a retrospective view of our D1 protein studies over the years. We gratefully acknowledge all of our collaborators whose names appear in the Chapter. In addition, we wish to acknowledge those who contributed to other aspects of D1 research in our laboratories: Adi Avni, Alessandra Cona, Bharat Chattoo, Martine Devic, Yoram Eyal, Robert Fluhr, Hillel Fromm, Maria Teresa Giardi, Richard B. Hallick, Dina Heller, Karl Jakob, Michael Koblizek, Vinod Kumar, Chiara Leonardi, Alexander Raskind, Judy St. John, and William Wergin.

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I. The Really Early Days

The D1 protein had its research debut in the mid 1970s. Several groups studying protein synthesis in isolated chloroplasts reported a membrane-associated polypeptide with an apparent molecular mass of 32 kDa on polyacrylamide gels. Eagelsham & Ellis (1974), working with pea chloroplasts at the University of Warwick, termed this protein "Peak D". Soon afterwards, it was found that chloroplast RNA could stimulate translation of an approximately 32 kDa protein in a heterologous in vitro system (Wheeler and Hartley, 1975). While this was unfolding in England, Arie Rosner, working in Jonathan Gressel's laboratory at the Weizmann Institute of Science in Israel, together with Daphna Sagher working in ours, reported a 0.5×10^6 Da chloroplast RNA fraction forming the bulk of the discrete pulse-labeled RNA molecules produced in vivo after transfer of steady-state dark grown *Spirodela* plants to light (Rosner et al., 1975). Actually, the biological system worked on in the Edelman laboratory at that time was *Euglena*, brought over from Jerome Schiff's Laboratory at Brandeis University. We mention this because shortly thereafter, Edelman left for a Sabbatical year in the United States. No sooner did he depart, than Avi Reisfeld, the other student in his group, impressed by the results obtained with *Spirodela*, quickly switched to the latter system and tied the 0.5×10^6 Da mRNA to the precursor of the 32 kDa protein in time to submit a paper to the first international plant molecular biology meeting, organized by Laurence Bogorad and Jacques Weil, in Strasburg, France in the Summer of 1976. To this day we are grateful to Reisfeld for his Israeli 'hutzpa' of switching systems when the boss was away. *Euglena* and *Spirodela* share a rare trait for photosynthetic organisms; both can grow indefinitely in the dark when supplied with an organic carbon source, thus enabling the study of chloroplast development from a state of true heterotrophy. Although *Euglena* is a zooflagellate of uncertain pedigree, *Spirodela* is a true blooded, if eccentric, monocot. In a series of articles, Reisfeld and others in the Edelman laboratory showed that the 32k Da polypeptide in *Spirodela* is the major membrane protein synthesized within the chloroplast (Edelman and Reisfeld, 1978), is derived from a rapidly synthesized 33.5 kDa precursor polypeptide (co-discovered in maize [Grebanier et al., 1978)]), and lacks lysine residues (Edelman and Reisfeld, 1980; Reisfeld et al., 1982). Soon afterwards, it was shown to occur universally and to be structurally similar in various photosynthetic organisms (Hoffman-Falk et al., 1982).

In one of the quirks of the field, the absence of lysine from the 32 kDa protein in *Spirodela* played an important role in the early days. In the summer meetings of 1982, Paul Whitfeld's group reported the very first sequence of the *psbA* gene (coding for the 32 kDa protein) in spinach and in tobacco, the model system of the time (Zurawski et al., 1982). When, at the end of their talk, they stated that the gene lacked codons for lysine, Charles Arntzen, who was chairing the session, jumped up and said "Edelman, you were right, no lysine." For several years following, the lack of lysine remained the criterion by which a chloroplast membrane protein was, or was not, deemed to be the 32 kDa protein. Indeed, the 32 kDa protein in most species investigated lacks lysine. However, in maize and some of the grains, lysine replaces arginine at codon 238.

II. Gernot Renger's Shield

Autar Mattoo arrived at the Weizmann Institute as a DAAD Scholar in 1979 to study chloroplast molecular biology, then an emerging discipline within plant molecular biology (Edelman et al., 1982 Preface). In the summer of 1980, Mattoo and a M.Sc. student, Hedda Hoffman-Falk, had succeeded in obtaining SDS-PAGE and electron transport results tying the 32 kDa protein to photosystem II (PS II) electron transport and herbicide sensitivity (Mattoo et al., 1981). Edelman was on annual army reserve duty when he received a field call from Mattoo. The message was terse: "The 32 kDa protein is the 'proteinaceous shield' of Renger!" The use of mild trypsin digestion to probe the structure-function relationship of surface exposed thylakoid membrane proteins (Regitz and Ohad, 1975) led Gernot Renger in 1976 to infer the existence of a "proteinaceous shield" covering the primary electron acceptor of PS II and acting as a regulator of electron flow between PS II and PS I (Renger, 1976). Mattoo found that mild trypsin treatment of *Spirodela* thylakoid membranes led to partial digestion of the 32 kDa protein. Under these conditions, photoreduction of ferricyanide becomes insensitive to diuron (DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea), the well-known inhibitor of PS II electron transport. When the thylakoids were preincubated with diuron, however, expression of insensitivity was prevented. The clincher came with the SDS-PAGE results, which brought on the phone call. Preincubation with diuron caused some conformational change in the 32 kDa protein that modified its trypsin digestibility and produced a different banding pattern