NDH-1 and NDH-2 Plastoquinone Reductases in Oxygenic Photosynthesis

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Abstract

Oxygenic photosynthesis converts solar energy into chemical energy in the chloroplasts of plants and microalgae as well as in prokaryotic cyanobacteria using a complex machinery composed of two photosystems and both membrane-bound and soluble electron carriers. In addition to the major photosynthetic complexes photosystem II (PSII), cytochrome b_6f, and photosystem I (PSI), chloroplasts also contain minor components, including a well-conserved type I NADH dehydrogenase (NDH-1) complex that functions in close relationship with photosynthesis and likewise originated from the endosymbiotic cyanobacterial ancestor. Some plants and many microalgal species have lost plastidial *ndh* genes and a functional NDH-1 complex during evolution, and studies have suggested that a plastidial type II NADH dehydrogenase (NDH-2) complex substitutes for the electron transport activity of NDH-1. However, although NDH-1 was initially thought to use NAD(P)H as an electron donor, recent research has demonstrated that both chloroplast and cyanobacterial NDH-1s oxidize reduced ferredoxin. We discuss more recent findings related to the biochemical composition and activity of NDH-1 and NDH-2 in relation to the physiology and regulation of photosynthesis, particularly focusing on their roles in cyclic electron flow around PSI, chlororespiration, and acclimation to changing environments.

Contents

1. INTRODUCTION

Oxygenic photosynthesis and respiration have long been considered independent mechanisms involving distinct electron transport chains, respectively located in two distinct compartments of eukaryotic cells, the chloroplasts and the mitochondria. The discovery of respiratory-like genes, enzymes, and complexes in chloroplasts of higher plants led scientists to revisit this paradigm. Oxygenic photosynthesis first appeared in cyanobacteria, prokaryotic cells in which photosynthetic and respiratory chains coexist and interact in the same cellular compartment. In higher-plant chloroplasts, as in cyanobacteria, the plastoquinone (PQ) pool serves as an electron buffer between photosystem II (PSII) and photosystem I (PSI). It can be reduced in a nonphotochemical manner by two different types of NAD(P)H PQ oxidoreductases, called type I NADH dehydrogenase (NDH-1) and type II NADH dehydrogenase (NDH-2). NDH-1 is a multisubunit complex similar to mitochondrial complex I, whereas NDH-2 is a single-subunit flavoenzyme. Compared with its mitochondrial counterpart, the NDH-1 complex has developed specific features and functions to cope with the chloroplast environment. Remarkably, in spite of a photosynthetic machinery resembling that of higher-plant chloroplasts, unicellular green algae lack a functional NDH-1 complex but have a plastidial NDH-2, which may substitute for the electron transport activity of NDH-1.

Our knowledge of the biogenesis, subunit composition, and regulation of the plastidial NDH-1 complex has greatly improved during the last decade, revealing an astonishing degree

of complexity, but the physiological function of this complex in higher plants remains obscure. Plastidial NDH-1 and NDH-2 are considered components of a complex network of regulatory mechanisms that allow the photosynthetic machinery to function optimally in fluctuating environmental conditions. The partial redundancy of these mechanisms likely explains the difficulty of identifying the physiological function of these enzymes.

2. PLASTIDIAL AND CYANOBACTERIAL NDH-1 COMPLEXES

The existence of a chloroplast NAD(P)H dehydrogenase complex was postulated from the sequencing of tobacco and liverwort plastid genomes, which revealed the presence of a set of 11 conserved genes (*ndh* genes) showing sequence homology with genes encoding subunits of mitochondrial NADH dehydrogenase (80, 96, 123). Experimental approaches combining biochemistry, genetics, bioinformatics, and proteomics helped to identify additional subunits of chloroplast NDH-1 encoded by the nuclear genomes of land plants (reviewed in 62, 120). In parallel, cyanobacteria genome sequencing and subsequent reverse genetics studies revealed the structural and functional multiplicity of cyanobacterial NDH-1 complexes (74, 93, 94, 118). In this section, we describe our present knowledge of the subunit composition of chloroplast and cyanobacterial complexes using the common subunit nomenclature proposed by Ifuku et al. (62).

2.1. Subunit Composition of the Plant NDH-1 Complex

The 11 plastid-encoded subunits (NdhA–K) are conserved in all NDH-related protein complexes and form an L-shaped skeleton. Chloroplast NDH-1 is a large protein complex consisting of these 11 subunits and more than 19 nucleus-encoded subunits (62). Based on the subunit composition of the NDH complex in different mutant backgrounds and on homologies with bacterial and mitochondrial NDH complexes, chloroplast NDH-1 was structurally subdivided into five subcomplexes: A, B, M (membrane), L (lumen), and ED (electron donor) (102, 146) (**Figure 1*a***).

Subcomplex A corresponds to the Q module of respiratory NADH dehydrogenases and includes four plastid-encoded subunits (NdhH–K). All the cofactors required for electron transport, from the soluble electron donor to the complex to PQ, are probably harbored by these subunits (50, 60). Chloroplast NDH-1 includes four additional nuclear-encoded subunits (NdhL–O), which copurify with a tagged NdhH subunit in tobacco (113). Subcomplex M consists of seven plastid-encoded subunits (NdhA–G) and forms the membrane arm that functions in proton translocation across the membrane (the P module in respiratory NADH dehydrogenase). Subcomplex B is composed of five subunits (PnsB1–5) and is specific to chloroplast NDH-1. PnsB4 and PnsB5 have transmembrane domains, whereas PnsB1, PnsB2, and PnsB3 are localized to the stroma side, probably anchored on PnsB4 and PnsB5. Although the molecular function of subcomplex B remains unelucidated, defects in its subunits result in the destabilization of the total complex (64, 102, 124, 128). Subcomplex L contains at least five subunits (PnsL1–5) and is also specific to chloroplast NDH-1. Phylogenetically, the occurrence of subcomplex L is linked to the formation of a supercomplex between NDH-1 and PSI (see Section 2.6). Three of the subcomplex L subunits (PnsL1–3) show sequence similarities to lumenal subunits of PSII: PnsL1 is PsbP-like protein 2 (PPL2) (63), and PnsL2 and PnsL3 are forms of PsbQ-like protein (PQL) (127, 145). In PSII, PsbP and PsbQ stabilize the PSII supercomplex by interacting with CP26 and CP47 (61), supporting the idea that subcomplex L stabilizes the NDH-1–PSI supercomplex at the lumen side.

Three subunits of subcomplex ED—NdhS, NdhT, and NdhU—have been identified by proteomic analysis of the NDH-1–PSI supercomplex (146). NdhS is involved in ferredoxin (Fd) binding (see Section 2.3). NdhT and NdhU are J and J-like proteins, respectively, that have a

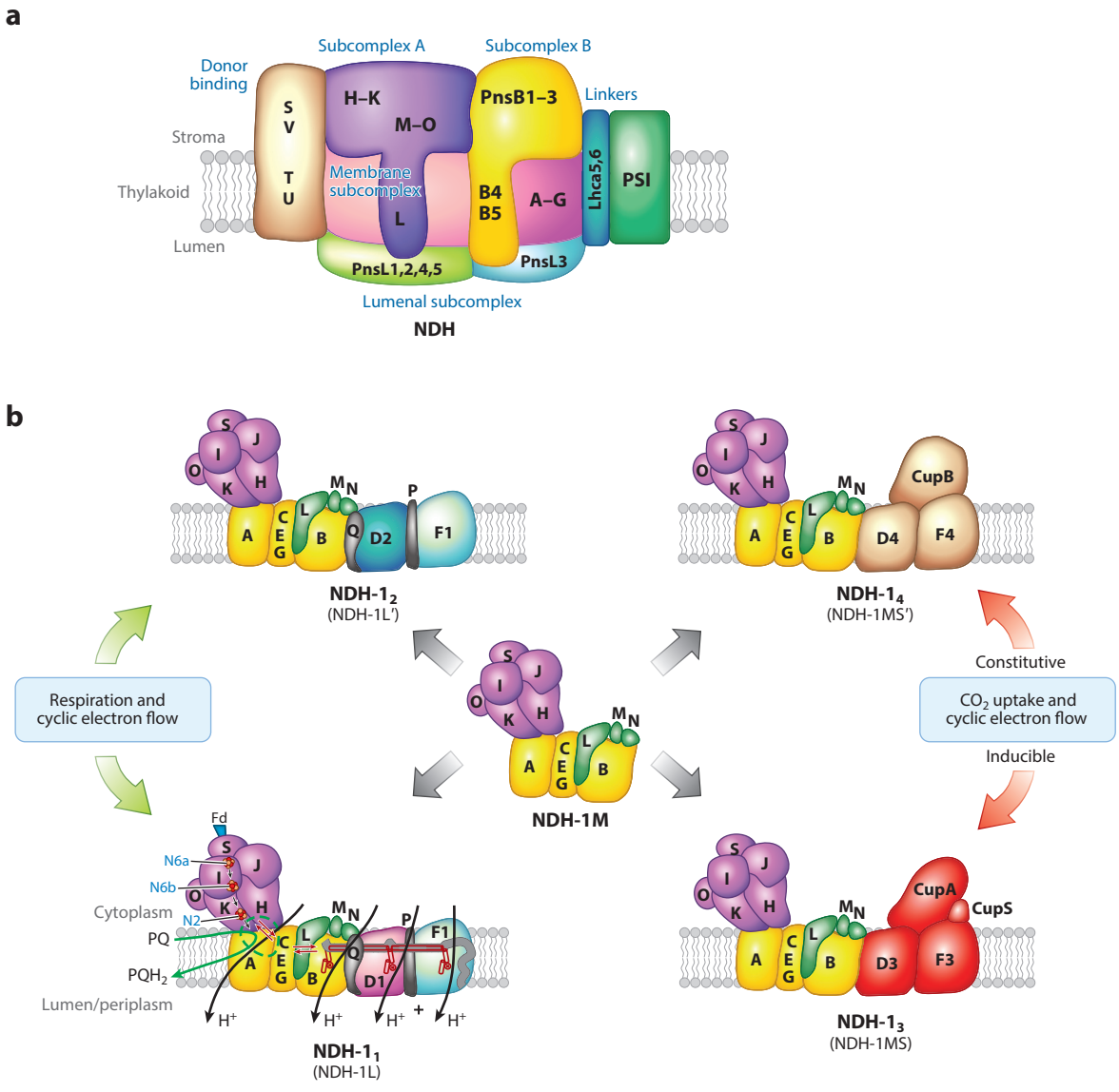

Figure 1

Subunit composition of (*a*) chloroplast and (*b*) cyanobacterial NDH-1 complexes. (*a*) The chloroplast model is based on an analysis of subunit stability in different mutant backgrounds (62) and the assembly model proposed by Peng et al. (101). This model does not provide information on the actual positions of subunits in the complex. (*b*) The cyanobacterial model is based on the crystal structure of respiratory complex I (8) and on a single-particle analysis of cyanobacterial NDH-1 (5). The two models differ in the positions of NdhM and NdhO (15, 120). Abbreviations: Fd, ferredoxin; PQ, plastoquinone; PQH$_2$, reduced plastoquinone; PSI, photosystem I.

transmembrane domain and likely form a heterodimer required for stabilizing NdhS (146). Fan et al. (35) recently identified NdhV as a new subunit loosely bound to subcomplex ED, forming the most fragile part of the complex. Subcomplex ED interacts with subcomplex A to form the Fd-binding site, which includes the Fd-oxidizing site (146).

2.2. Subunit Composition of Cyanobacterial NDH-1 Complexes

Proton motive force: the force generated by electron transport reactions acting as a proton pump

The development of blue native gel electrophoretic separation of thylakoid protein complexes combined with mass spectrometric identification of protein subunits led to the characterization of different NDH-1 complexes in the cyanobacterial thylakoid membrane (9, 54, 95, 144, 149, 150). All of these complexes contain the NDH-1M module, which is composed of both hydrophilic and hydrophobic domains and is presently known to comprise 14 subunits (NdhA–C, NdhE, NdhG–O, and NdhS) (**Figure 1*b***). NDH-1M has no physiological function by itself but represents an assembly intermediate for functional NDH-1 complexes (9). The NdhH–K, NdhO, and NdhS subunits form the hydrophilic domain, while the NdhA–C, NdhE, NdhG, and NdhL–N subunits are components of the hydrophobic membrane domain (9). The NdhO subunit, earlier assigned to the hydrophobic domain (9), was recently shown to strongly interact with the NdhI and NdhK subunits of the hydrophilic domain, thereby providing flexibility and maximal NDH-dependent cyclic electron transport (NDH-CET) activity under high-light conditions (151). The NdhS subunit of the hydrophilic domain of NDH-1M is essential for binding of Fd, the putative electron donor to cyanobacterial NDH-1 complexes (9), as in the case of plant chloroplasts (see Section 2.3).

Whereas assembly mechanisms of the chloroplast NDH-1 complex are now quite well described (see Section 2.4), very little is known about the assembly of NDH-1M. So far, only one maturation factor, the Slr1097 (CRR6) protein, has been identified in *Synechocystis* sp. PCC6803 (27). In addition to the NDH-1M module, which is common to all complexes, cyanobacterial NDH-1 complexes differ in the nature of the NdhD and NdhF subunits. The *Synechocystis* sp. PCC6803 genome contains six different *ndhD* genes (*ndhD1–6*) and three different *ndhF* genes (*ndhF1*, *ndhF3*, and *ndhF4*).

2.2.1. The NDH-1_1 and NDH-1_2 complexes. NDH-1_1 (also called NDH-1L) is present in cyanobacterial thylakoid membranes as a 450-kDa protein complex (54). In addition to the NDH-1M module, NDH-1_1 has two specific subunits, NdhD1 and NdhF1, that extend the membrane domain and give the complex an L shape that is typical of bacterial or mammalian NDH-1 complexes (for a review, see 9). Studies have recently shown that the NDH-1_1 complex includes two small subunits, NdhP and NdhO, that are localized to the membrane arm and are essential for the stabilization and optimal activity of the complex (117, 142, 152).

The NDH-1_2 complex (also called NDH-1L′) harbors the NdhD2 subunit instead of NdhD1. NDH-1_2 may be expressed in particular environmental conditions, as expression of the *ndhD2* gene differs conspicuously from that of the *ndhD1* gene, increasing in particular upon CO_2 limitation (140) or iron depletion (53). A recent study suggested that this complex withdraws the excess of electrons in the intersystem chain by catalyzing reverse electron flow using the proton motive force and reduced PQ (PQH_2) to reduce $NAD(P)^+$ or Fd (B. Forberich, S. Künzel, L. Cournac, Y. Allahverdiyeva, R. Schulz, et al., manuscript in review).

2.2.2. The NDH-1_3 and NDH-1_4 complexes. The NDH-1_3 complex (also called NDH-1MS) contains NdhD3 and NdhF3 and two additional subunits, CupA and CupS, that are bound to the NdhD3 and NdhF3 proteins in the distal membrane arm of the complex (**Figure 1*b***). Upon isolation, the NDH-1_3 complex falls into two parts in the blue native gels, the NDH-1M complex and a small NDH-1S subcomplex (54, 149). Isolation of the complex from a thermophilic *Thermosynechococcus elongatus* cyanobacterium demonstrated that these subcomplexes are part of the larger NDH-1_3 complex (150). The NDH-1_4 complex (also called NDH-1MS′) contains NdhD4, NdhF4, and CupB subunits in addition to the NDH-1M complex (142).

2.3. The Nature of the Electron Donor to NDH-1

Compared with the minimum set of 14 Nuo subunits of the *Escherichia coli* NADH dehydrogenase (44), plastid genomes lack three important genes encoding subunits (corresponding to bacterial NuoE, NuoF, and NuoG) that form the NADH-oxidizing (N) module. Missing genes were not found in land-plant nuclear genomes or in cyanobacterial genomes, and Friedrich et al. (44) proposed that chloroplast and cyanobacterial NDH-1 are equipped with an NAD(P)H-oxidizing module different from that present in bacterial or mitochondrial complex I. The nature of the soluble electron donor to the plastidial NDH-1 complex has been a matter of debate. NAD(P)H-dependent PQ reduction activities have been measured in potato and spinach thylakoid membranes (22, 32). However, the comparison of NAD(P)H oxidation activities of thylakoid membranes isolated from wild-type and mutant *Arabidopsis* plants defective in chloroplast NDH-1 showed no statistically significant difference (124). Purification of the enzyme is the most straightforward strategy to determine the complex activity, but the low amount and fragility of the plastidial NDH-1 complex make this approach difficult. An ~550-kDa complex isolated from pea leaves and composed of at least 16 subunits catalyzes NADH oxidation, but the specific activity of the complex was much lower than is usually measured for NADH dehydrogenases (115). NADH-oxidizing activity was also reported in a histidine-tagged NDH-1 complex purified from Ni^{2+} affinity chromatography (113).

In *Arabidopsis* ruptured chloroplasts, NADPH-dependent PQ reduction by the NDH-1 complex is strictly dependent on the presence of Fd (86). Proteomic analysis of the NDH-PSI supercomplex identified a novel NDH subunit (NdhS/CRR31) involved in high-affinity binding of Fd (104, 146). NdhS holds an Src homology 3 domain–like fold with a tertiary structure similar to the Fd-binding site of the PSI subunit PsaE. A positive surface charge of the pocket in the Src homology 3 domain–like fold is essential for electrostatic interaction with Fd (147). NdhS is conserved in cyanobacteria and has a similar function in *Synechocystis* sp. PCC6803 (11). He et al. (50) recently affinity purified the NDH-1_1 complex of *T. elongatus* via a histidine-rich region naturally present in NdhF1. The purified complex contained 14 NDH-1 subunits, including NdhS, and protein interactions between NdhS and Fd were confirmed by surface plasmon resonance analysis. As with the chloroplast NDH-1 complex, cyanobacterial NDH-1_1 is likely to accept electrons from Fd, and the NdhS subunit forms the Fd-binding site (11, 50).

Although pioneering studies reported an NADH-oxidizing activity of plastidial NDH-1, most recent studies performed in *Arabidopsis* and cyanobacteria have concluded that photosynthetic NDH-1 complexes accept electrons from Fd rather than from NADH or NADPH. Early results showing an NADH dehydrogenase activity of the NDH-1 complex might have been due to the presence of contaminating enzyme activities. The chloroplast NDH-1 should then be considered an Fd-PQ reductase rather than a genuine NAD(P)H dehydrogenase.

2.4. Biogenesis of NDH-1

Different proteins have been identified as auxiliary components involved in NDH-1 biogenesis (**Table 1**). Assembly of subcomplex A proceeds in the stroma of chloroplasts in a manner similar to that described in human mitochondria (101). In mitochondria, NDUFS2 (corresponding to NdhH) and NDUFS3 (NdhJ) initiate Q module assembly (137). Subsequently, NDUFS7 (NdhK), NDUFS8 (NdhI), and NDFUA9 are incorporated into this assembly intermediate, which is followed by an interaction with ND1 (NdhA) in the mitochondrial inner membrane to form an ~400-kDa assembly intermediate (84). In thylakoid membranes, any defect in subcomplex A subunits almost completely destabilizes subcomplex A.

Table 1 Assembly factors of NDH-1

	Motif	Function	Reference
CRR1	NAD(P)H-binding	Assembly of NdhK or NdhM	122
CRR6	None	Assembly of NdhI	100
CRR7	None	Insertion of subcomplex A into the membrane part	100
CRR41	None	Scaffold for subcomplex assembly	101
CRR42	None	Transition from NAI500 to NAI400	101
Cpn60β4	Minor chaperonin β	Folding of NdhH	103
PAM68L	None	Assembly of the membrane part	4

Based on the accumulation pattern of assembly intermediates in different mutant backgrounds, Peng et al. (106) proposed a model of the assembly process for subcomplex A. In an *Arabidopsis* mutant (*crr27*) defective in Cpn60β4, none of the assembly intermediates are detected in the stroma (101). Cpn60β4 is involved in the folding of NdhH, suggesting that NdhH initiates the assembly of subcomplex A in chloroplasts (103). After folding, NdhH is incorporated into the ~500-kDa NDH-1 assembly intermediate (NAI500), which includes NdhO and CRR41 (101). NdhO may directly interact with NdhH. CRR41 is a nonsubunit factor that is required for the assembly of subcomplex A but ultimately absent in the NDH-1–PSI supercomplex (101). NdhH, NdhO, and CRR41 are necessary to stabilize each other. The order of subsequent incorporation of NdhI–K and NdhM into NAI500 to form NAI400 is not well established. NdhJ stably accumulates in the stroma of *crr1* mutants lacking NdhK, NdhM (122), or NdhI (100). By contrast, NdhJ is unstable in mutants (*crr27*, *ndho*, and *crr41*) that are defective in NAI500 accumulation (101). As in the human mitochondria, NdhJ (NDUFS3) may interact with NdhH (NDUFS2) in an early step of subcomplex A assembly.

CRR6 was first identified by classical genetics and then further elucidated by an HA-epitope strategy aiming at purifying the assembly intermediates of subcomplex A (100, 101). CRR6 copurified with nonsubunit assembly factors (CRR1, CRR41, CRR42, and HCF101) as well as NDH subunits (NdhH–K, NdhM, and NdhO). Accumulation of NdhI in the stroma of the *crr6* mutant was impaired, suggesting that CRR6 is required for the incorporation of NdhI into NAI500. However, CRR6 was not detected in any NAIs in clear native gel, suggesting that CRR6 transiently interacts with NAIs via NdhI.

CRR1 was also discovered by classical genetics as a mysterious homolog of dihydrodipicolinate reductase that functions in lysine biosynthesis (122). Although the molecular function of CRR1 is unclear, it is essential for the accumulation of NdhK (101). Because NdhM is essential for stabilizing NdhK in the stroma, it is also possible that CRR1 is required for the accumulation of NdhK via the stabilization of NdhM.

CRR42 was identified in the coimmunoprecipitates with CRR6 (101). Because NdhN was not detected in coimmunoprecipitates with CRR42, CRR42 is likely released from NAI400 before the incorporation of NdhN (101). Subcomplex A is almost fully assembled in the stroma and then interacts with NdhL and probably also with NdhA in the thylakoid membrane. This process is also conserved in the mitochondrial NADH dehydrogenase on which the fully assembled Q module interacts with ND1 in the mitochondrial inner membrane (84). As discussed below, NDH-1-related complexes evolved by combining different preexisting modules (42). Assembly of the complex likely proceeds in each module by putting together all the modules in the membrane. The assembly of complex I–related enzymes may follow the evolutionarily conserved scenario.

Although the assembly process of subcomplex A is well documented, little is known about the assembly of the membrane embedded arm (subcomplexes B and M) of the chloroplast NDH-1 complex. In human mitochondria, the ~400-kDa intermediate, which includes DUFS2, -3, -7, -8, and -9, interacts with an ~460-kDa intermediate that includes ND2 (corresponding to NdhB), ND3 (NdhC), ND4L (NdhE), and ND6 (NdhG) to form an ~650-kDa intermediate. Subsequently, ND4 (NdhD) and ND5 (NdhF) are fused to the most peripheral part of the membrane arm to form an ~830-kDa intermediate (137). This assembly process may be conserved in photosynthetic NDH-1, because NdhD and NdhF are exchangeable to form the NDH-1 complexes with different functions in cyanobacteria (9). Finally, the ~830-kDa intermediate is equipped with the N module to form the ~980-kDa mature complex (137). This final process is missing in photosynthetic NDH-1 complexes, which lack the N module.

Interestingly, biogenesis of PSII and NDH-1 plastidial complexes show some similarity. Two closely related *Arabidopsis* proteins, PHOTOSYNTHESIS AFFECTED MUTANT 68 (PAM68) and PAM68-LIKE (PAM68L), are involved in the assembly of the PSII core (4) and of the membrane part of chloroplast NDH-1 (3), respectively.

2.5. Regulation of NDH-1

Although molecular mechanisms of distinct assembly steps of NDH-1 have been widely elucidated based on the discovery of specific mutants, information is still lacking on how each step is orchestrated to regulate the biogenesis of the complex. RNA editing is a process that alters genetic information in RNA molecules and frequently occurs in the plastids and mitochondria of land plants (130). Remarkably, 16 of the 34 editing sites in the *Arabidopsis* plastid genome are associated with four *ndh* genes (*ndhB*, *ndhD*, *ndhF*, and *ndhG*) (119). An intriguing question is why the distribution of editing sites in the plastid genome is biased in this way. RNA editing is considered to be a system of "genome debugging" (75), which is unlikely to have a regulatory role in plastid gene expression. The physiological meaning of RNA editing of *ndh* genes is still unclear, but it may have conferred genetic diversity to NDH-1 subunits during the evolution of land plants (119).

Induction of NDH-related genes under certain conditions would be a hint to predict the physiological function of chloroplast NDH-1. However, although tobacco plants lacking NDH-1 were reported to be sensitive to several environmental stresses, stress conditions did not induce expression of NDH-1-related genes in *Arabidopsis*. Although the presence of a functional chloroplast NDH-1 is required for optimal growth in an *Arabidopsis proton gradient regulation 5* (*pgr5*) mutant background, the level of NDH-1 complex is not higher in this background than in a wild-type background (86). Nonetheless, public microarray data suggest that NDH-1-related genes form several coexpression groups, which enabled Takabayashi et al. (128) to identify novel subunits. A sigma factor (Sig4) is specifically required for the transcription of *ndhF* (38), and coexpression analysis [using the ATTED-II database, version 7 (92)] indicated that the transcriptional profile of the *sig4* genes is related to that of *CRR7* genes. Transcript levels of NDH-1-related genes are downregulated in *Arabidopsis* genotypes with reduced levels of ascorbate or glutathione or higher levels of hydrogen peroxide (H_2O_2) (111), although H_2O_2 was recently proposed to activate NDH-CET (126). Plastid NDH-1 levels were also reduced following perception of a pathogenic cue (46).

A few reports have proposed that chloroplast NDH-1 activity could be regulated by posttranslational modifications (phosphorylation or redox modification) of some of its subunits. Martin et al. (77) proposed that phosphorylation of the NdhF subunit regulates NDH-1 in response to oxidative stress. Courteille et al. (26) proposed a redox regulation of NDH-1 on the basis that the

growth phenotype of the *pgr5* mutant was suppressed in a double mutant lacking thioredoxin m4. Further work remains to fully characterize posttranslational modifications of the NDH-1 complex and determine their physiological importance.

Cyclic electron flow (CEF): a pathway of electrons around photosystem I enabling the generation of additional proton motive force and ATP

CEF supercomplex: a complex formed by molecular interactions between the PSI supercomplex, the cytochrome b_6f complex, and other components of CEF in *Chlamydomonas*

2.6. Involvement of NDH-1 in a Supercomplex with Photosystem I

In *Arabidopsis*, chloroplast NDH-1 forms a cyclic electron flow (CEF) supercomplex with PSI (105). The formation of this supercomplex is intermediated by two minor light-harvesting complex I (LHCI) proteins, Lhca5 and Lhca6 (102), and is required for the stabilization of NDH-1, especially under high-light conditions (104). Recently, single-particle electron microscopy analysis of the supercomplex showed that two copies of PSI are attached to one copy of NDH-1, with LHCI proteins being involved in the attachment (70). The ability to form a supercomplex between NDH-1 and PSI likely represents a relatively recent evolutionary acquisition, as genes encoding Lhca5 and Lhca6 are not found in *Marchantia polymorpha*. There is presently no experimental evidence for formation of NDH-1L–PSI supercomplexes in cyanobacteria, and in *Marchantia*, NDH-1 occurs as a monomer (136). *Physcomitrella patens* falls in the middle: It holds a single LHC1 protein related to Lhca5, and only a part of NDH-1 forms a supercomplex with PSI (3). The formation of a supercomplex between PSI and NDH-1 may allow a more efficient channeling of electrons from PSI to NDH-1, thereby improving CEF, but experimental evidence for such a role is still lacking. In cyanobacteria, the NdhP subunit specific to the NDH-1_1 complex improves CEF efficiency by mediating a coupling with PSI (117), and it may have a similar function in facilitating the channeling of electrons between the two complexes.

3. PLASTIDIAL AND CYANOBACTERIAL NDH-2s

NDH-2s are single-subunit flavoenzymes that bypass the activity of complex I in plant mitochondria, yeasts, and some bacteria (82). Peltier & Cournac (98) proposed that NDH-2 replaces the electron transfer activity of NDH-1 in species such as microalgae, which lack the plastidial NDH-1 complex. NDH-2s are nonelectrogenic and monomeric enzymes of approximately 50 kDa, anchored to membranes and harboring two β sheet–α helix–β sheet (Rossmann fold) domains, one involved in the binding of flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN) and the other involved in the binding of NAD(P)H (82). The crystal structures of yeast Ndi1 and bacterial NDH-2s were recently solved, showing a dimeric organization, a membrane-anchoring domain, and binding pockets for FAD, ubiquinone, and NADH, but the enzyme mechanism remains unclear (39, 52).

The only chloroplast NDH-2 characterized so far at the enzyme level is the *Chlamydomonas* Nda2 (30). Recombinant CrNda2 can reduce PQs by using NADH or NADPH as an electron donor, with NADH being the preferential substrate (30). The substrate specificity of NDH-2s is determined by the nature of a residue located at the end of the NADH-binding domain (29). Enzymes showing a preference for NADH harbor an acidic residue, a basic residue present in enzymes with a higher NADPH activity. The preference for NADH as a substrate is an intriguing feature for a plastidial enzyme because NADPH, rather than NADH, is considered the major reduced nucleotide species present in this cellular compartment (51). This might be related to the presence of a transhydrogenase in algal chloroplasts (132), an enzyme interconverting NADH and NADPH species. In contrast to most NDH-2s, which use FAD as a non-covalently-bound cofactor, CrNda2 uses FMN (30). The presence of an FMN cofactor is a rare feature of NDH-2s that has so far been documented in a few enzymes, including one from an archaeon (7) and another from the protozoan *Trypanosoma brucei* (36).

Chlororespiration: an electron transport chain present in chloroplasts that involves nonphotochemical reduction and oxidation of plastoquinones and reducing O_2

Plastid terminal oxidase (PTOX): the chloroplast terminal oxidase of chlororespiration, which oxidizes plastoquinols and reduces O_2

The genomes of photosynthetic eukaryotes contain several NDH-2 genes: *Chlamydomonas reinhardtii* (30) and *Physcomitrella patens* (143) each have six, and *Arabidopsis thaliana* has seven (83). *Synechocystis* sp. PCC6803 has three genes encoding NDH-2s (59). In photosynthetic eukaryotes, some of these NDH-2s are targeted to mitochondria or peroxisomes, whereas others are plastidial. Dual targeting has been shown for several NDH-2s in *Physcomitrella* and *Arabidopsis* (143). Based on homologies with bacterial and fungal sequences, plant NDH-2s have been classified into three distinct subgroups, called NDA, NDB, and NDC. Whereas NDA and NDB are related to fungal sequences, NDC is related to cyanobacterial NDH-2s (83). In *Chlamydomonas*, CrNda2 and CrNda3, which belong to the NDB group, are targeted to chloroplasts (30, 65, 132). In *Physcomitrella*, three NDH-2s are targeted to the chloroplast, including two from the NDB group and one from the NDC group (143). In *Arabidopsis*, only one of the seven NDH-2s, the cyanobacterial-type NDC, is targeted to chloroplasts (143). All of the plastidial NDH-2s classified so far belong to the NDB or NDC group.

In yeasts, bacteria, and plant mitochondria, NDH-2s are involved in the respiratory electron transport chain. In organisms lacking a functional NDH-1, such as *Saccharomyces cerevisiae*, NDH-2s are the only enzymes of the respiratory chain able to oxidize NADH (82). What is the function of NDH-2 in chloroplasts and cyanobacteria? Do these enzymes replace the electron transport activity of NDH-1 in species lacking the plastidial complex, such as microalgae, or are these enzymes involved in specific metabolic functions? We discuss these questions in the following sections.

4. METABOLIC AND PHYSIOLOGICAL FUNCTIONS OF NDH-1 AND NDH-2

Since the discovery of conserved plastid genes encoding a functional NDH-1 complex in land plants, genetic and biochemical studies have led to the identification of a large set of nuclear genes involved in the regulation, biogenesis, and structure of the NDH-1 complex. However, although NDH-1 was proposed initially to be involved in chlororespiration and later to be involved in CEF, its physiological function has long remained elusive.

4.1. Chlororespiration and Cyanobacterial Respiration

The presence of an NDH-1 complex in chloroplasts was initially viewed as support for the concept of chlororespiration (96, 97), a respiratory chain previously seen in microalgal chloroplasts (13) (**Figure 2*a*,*b***). However, it turned out that microalgal chloroplasts do not harbor *ndh* genes and have no functional NDH-1 complex (114). This contradiction was apparently resolved when an NDH-2 called CrNda2 was discovered in *Chlamydomonas* chloroplasts (30, 65, 88), as it was assumed that NDH-2 may functionally replace NDH-1. In higher-plant chloroplasts, studies suggested that the NDH-1 complex participates in the chlororespiratory electron transport pathway (19, 98) by reducing the PQ pool from NAD(P)H, with PQH_2 oxidized by a plastid terminal oxidase (PTOX) directly branched to the PQ pool (25, 58).

However, the recent finding that reduced Fd is the electron donor to the complex (104, 146) argues against an involvement of NDH-1 in a respiratory activity on the model of mitochondrial or bacterial respiration. Indeed, respiratory electron transport chains use NAD(P)H produced by metabolic reactions as electron donors. Reduced Fd is produced in light by the photosynthetic electron transport chain. In chloroplasts, Fd-NADP reductase (FNR) functions in reducing $NADP^+$ from Fd, but specific FNR enzymes such as root-type FNR have a lower negative midpoint potential that facilitates the reduction of Fd from NADPH (90). Such FNRs are involved in

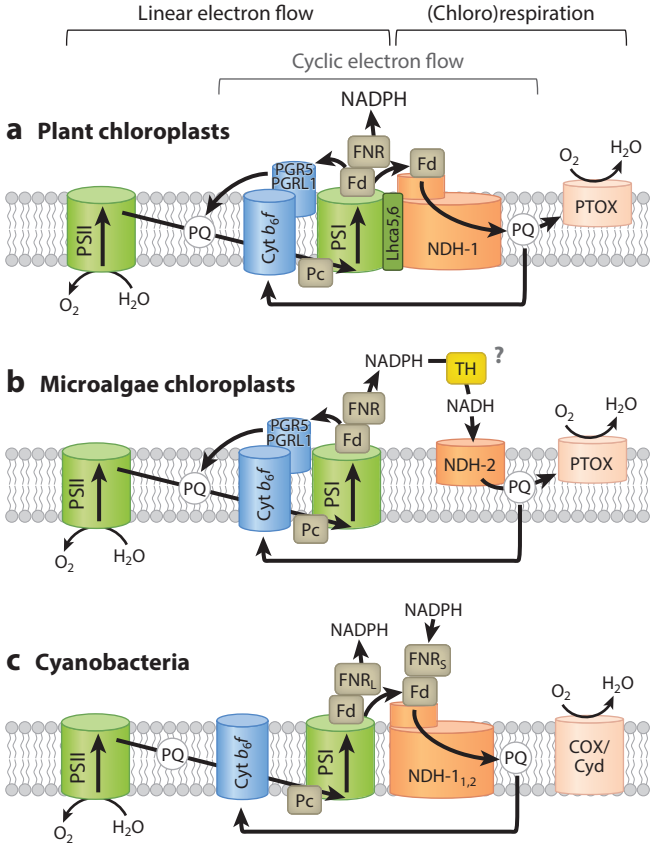

Figure 2

Electron transfer pathways involving NDH-1 and NDH-2 in oxygenic photosynthesis in (*a*) plants, (*b*) microalgae, and (*c*) cyanobacteria. Electron transfer pathways related to linear electron flow, cyclic electron flow, and (chloro)respiration are shown, based on experimental results obtained in *Arabidopsis thaliana* for plant chloroplasts, *Chlamydomonas reinhardtii* for microalgae, and *Synechocystis* sp. PCC6803 for cyanobacteria. Abbreviations: COX, cytochrome aa_3 oxidase; Cyd, cytochrome *bd* quinol oxidase; Cyt b_6f, cytochrome b_6f complex; Fd, ferredoxin; FNR, ferredoxin-NADP reductase; FNR_L, large FNR isoform; FNR_S, small FNR isoform; Pc, plastocyanin; PGR5, PROTON GRADIENT REGULATION 5; PGRL1, PGR5-Like Photosynthetic Phenotype 1; PQ, plastoquinone pool; PSI, photosystem I; PSII, photosystem II; PTOX, plastid terminal oxidase; TH, transhydrogenase.

nitrate assimilation in roots and may participate in supplying the NDH-1 complex in reductants, provided they would be expressed in photosynthetic plastids.

Alternatively, NDH-2s, which use NAD(P)H as an electron donor, could be involved in chlororespiration. However, the only NDH-2 identified so far in angiosperms is associated with plastoglobuli and involved in prenylquinone metabolism (108). Therefore, the existence of a complete chlororespiratory chain from NAD(P)H to O_2 is doubtful in land plants and might be restricted to organisms harboring a specific FNR able to reduce Fd from NADPH, or to organisms, such as microalgae, that harbor a plastidial NDH-2 involved in PQ reduction. It is worth mentioning here that, in any case, chlororespiration should not be considered a pure respiratory electron transport chain that converts reducing power into phosphorylating power, because in contrast to respiratory chains, which contain electrogenic complexes, both NDH-2 and PTOX

are nonelectrogenic enzymes (89). Therefore, chlororespiration should be viewed as a futile pathway involved in the dissipation of energy or as a regulatory mechanism participating in the poising of intersystem electron carriers.

PGR5 and PGRL1: two essential components of the antimycin A–sensitive pathway of CEF

In cyanobacteria, photosynthesis and respiration are located in the same cellular compartment and share the same intersystem electron carriers, such as the PQ pool and the cytochrome b_6f complex (116). Different enzymes, including NDH-1, NDH-2, and succinate dehydrogenase, are likely involved in the dark reduction of the PQ pool. Low respiration rates and impairment of photoheterotrophic growth were reported in the *Synechocystis* sp. PCC6803 Δ*ndhB* mutant (M55), which lacks all of the NDH-1 complexes (93), as well as in double mutants inactivated in both the *ndhD1* and *ndhD2* genes, and it was concluded that the NDH-1_1 and NDH-1_2 complexes are involved in respiration (94). More recent data have verified a major role for the NDH-1_1 complex in cyanobacterial respiration (see 9), and active electron flow from metabolites to the PQ pool was indeed strongly suppressed upon depletion of the NdhF1 subunit (14). Similarly, the absence of other NDH-1_1- and NDH-1_2-specific subunits (either NdhP or NdhQ, which stabilize the complex) drastically decreases the respiratory capacity of cyanobacterial cells (117, 152).

Because cyanobacterial NDH-1_1 and NDH-1_2 complexes, like chloroplast NDH-1, use reduced Fd as an electron donor, the involvement of NDH-1 in respiration would require a specific FNR involved in the production of reduced Fd. The *Synechocystis* sp. PCC6803 genome includes a single FNR-encoding gene, but two FNR isoforms [the large (FNR_L) and small (FNR_S) isoforms] are produced from this single gene (133). FNR_L is involved in $NADP^+$ reduction and photosynthesis, and FNR_S, which accumulates during heterotrophic growth, catalyzes Fd reduction from NADPH and likely participates in respiration, particularly in conditions of high cellular energy status (133).

4.2. Cyclic Electron Flow Around Photosystem I

CEF around PSI is an important reaction of oxygenic photosynthesis that contributes to increasing the proton gradient and producing ATP in the photosynthetic electron transport chain to match metabolic needs (67) (**Figure 2**). CEF was initially considered an antimycin A–sensitive reaction involving a specific component called Fd-PQ oxidoreductase (21). The involvement of NDH-1 in CEF was proposed based on knockouts of the plastidial complex first in tobacco (19, 121) and then in *Arabidopsis* (49). Physiological studies performed on tobacco plants with an inactivated NDH-1 complex concluded that two pathways of CEF operate around PSI: one involving the NDH-1 complex and one, sensitive to antimycin A, involving Fd-PQ reductase (66). Further genetic studies identified PGR5 (87) and PGR5-Like Photosynthetic Phenotype 1 (PGRL1) (28) as essential components of the antimycin A–sensitive pathway, and Hertl et al. (55) recently proposed that PGRL1 acts as an Fd-PQ reductase. The recent discovery that reduced Fd is the electron donor to the NDH-1 complex (104, 146) led to the conclusion that this complex is an antimycin A–insensitive Fd-PQ reductase. Although mutants only affected in the NDH-1 complex show no obvious change in their growth phenotype, the growth of mutants defective in both CEF pathways (NDH-1 and PGR5/PGRL1) is severely impaired, indicating that these pathways can complement each other (86). Given the low abundance of NDH-1 in thylakoid membranes and the absence of a growth phenotype in mutants lacking NDH-1, NDH-1 is generally considered to make a minor contribution to CEF. However, studies of NDH-1 knockout mutants concluded that NDH-1 may significantly contribute to the proton motive force in low light (136, 148), whereas the PGR5/PGRL1-dependent pathway would preferentially function at high light intensities (139).

Two CEF pathways also operate in *Chlamydomonas* (112), one involving PGR5/PGRL1 (68, 134) and the other involving CreNda2 (6, 65) (**Figure 2*b***). Because CrNda2 uses NADH as a

preferential substrate (29), the latter likely requires an interconversion of NADPH (produced by photosynthesis) into NADH, which might be carried out by a transhydrogenase present in *Chlamydomonas* chloroplasts (132). Based on measurements of the maximum capacities of these two pathways, Alric (1) concluded that the PGR5/PGRL1 pathway is the major contributor under reducing conditions.

Cyanobacteria lack the PGR5/PGRL1 CEF components (99), and CEF relies on the presence of NDH-1 complexes (9). Two types of CEF pathways relying on NDH-1 complexes have been distinguished in cyanobacteria: one related to the NDH-1_1 and NDH-1_2 complexes, which also participate in respiration and heterotrophic growth (see Section 4.1), and one related to the NDH-1_3 and NDH-1_4 complexes, which are involved in the CO_2-concentrating mechanism (CCM) (see Section 4.4) (14). The possible function of cyanobacterial NDH-2s in CEF remains to be elucidated.

CO_2-concentrating mechanism (CCM): a mechanism that enables the concentration of inorganic carbon in the vicinity of Rubisco, the carboxylating enzyme of photosynthesis

In addition to supplying extra ATP for photosynthetic CO_2 fixation, CEF pathways cooperate to generate a proton motive force that may trigger important regulatory mechanisms of photosynthesis. When CO_2 fixation and ADP regeneration are limited (e.g., in stress conditions), the CEF-dependent proton motive force is used to induce nonphotochemical quenching and photosynthetic control at the cytochrome b_6f level (41).

4.3. Putative Role as Redox Sensors

Given the low abundance of NDH complexes in chloroplast membranes, these complexes may have a regulatory function (98, 113). In *Synechocystis*, although PSI-deficient mutants are sensitive to high light, inactivation of one or several NDH-2s allowed recovering growth under high-light conditions, leading Howitt et al. (59) to conclude that cyanobacterial NDH-2s are not involved in respiration and instead act as sensors of the redox state of the PQ pool. The presence of FMN as a cofactor in CreNda2, the plastidial NDH-2 identified in *Chlamydomonas*, may have physiological implications because FMN catalyzes one-electron transfer reactions known to produce reactive oxygen species (ROS) (36). The majority of ROS produced by NDH-1s originates from FMN, with this production occurring when the quinone reductase site is blocked (12). The *Chlamydomonas* CrNda2 belongs to the NDB group of NDH-2s, which harbor a putative EF-hand Ca^{2+}-binding domain (30). *Arabidopsis* NDB1 and NDB2 bind Ca^{2+} (47), but these enzymes are located in mitochondria, not in plastids (143). Whether plastidial NDH-2s are regulated by binding Ca^{2+} remains to be elucidated. Terashima et al. (131) reported that a Ca^{2+} sensor regulates the PGRL1-dependent CEF pathway in *Chlamydomonas*. Therefore, Ca^{2+} might regulate the activity of both PGRL1- and NDH-2-mediated CEF pathways. Based on a phosphoproteome survey of the *Chlamydomonas* eyespot, Wagner et al. (138) identified Nda2 as a highly phosphorylated protein, thus indicating that the enzyme activity is subject to strong posttranslational regulations. Taken together, these data indicate that plastidial NDH-2 might be subject to strong regulations, which may have a signaling function in relation to Ca^{2+} binding and ROS production. As suggested for cyanobacterial NDH-2s, it is possible that plastidial NDH-2s are sensors of the PQ pool redox state.

4.4. CO_2-Concentrating Mechanisms of Cyanobacteria and C_4 Plants

Cyanobacteria have evolved a CCM that greatly improves photosynthetic performances and growth under CO_2-limiting conditions (110). By studying a *Synechocystis* sp. PCC6803 mutant that had an inactive *ndhB* gene and required high CO_2 concentrations for growth, Ogawa (93) established a link between the CCM and NDH-1. A similar high-CO_2-requiring phenotype was

observed in response to inactivation of both *ndhD3* and *ndhD4*, thus showing that two NDH-1 complexes, NDH-1_3 and NDH-1_4, participate in the cyanobacterial CCM (74, 94). The CupA and CupS subunits are involved in CO_2 conversion to bicarbonate (HCO_3^-) (74, 110), while other subunits of the complex supply energy to the CO_2-pumping mechanism by performing CEF (14) and producing ATP. NDH-1_3 is an inducible low-affinity CO_2 uptake mechanism that is absent when cyanobacteria grow at elevated CO_2 concentrations (1–5%) but rapidly accumulates in thylakoid membranes upon CO_2 deprivation (10, 69). NDH-1_4 is a constitutive low-affinity CO_2 uptake system. In *Synechocystis*, NDH-1_4 is still elusive at the protein level, but Wulfhorst et al. (142) recently found the NDH-1_4-specific subunits NdhD4, NdhF4, and CupB in the *T. elongatus* thylakoid membrane. Based on the presence of conserved *ndhD3/ndhF3* and *ndhD4/ndhF4* genes, many cyanobacterial CCMs appear to depend on functional NDH-1_3 and NDH-1_4 complexes (110). However, some cyanobacterial species (such as *Prochlorococcus*) and microalgae (such as Chlorophyceae), despite harboring efficient CCMs (56, 141), lack specific components of the NDH-1_3 and NDH-1_4 complexes (110) or lack a functional NDH-1 complex. Therefore, depending on the species, the functioning of the CCM relies on different mechanisms.

C_4 plants have evolved a CCM that improves CO_2 fixation by limiting the oxygenase activity of Rubisco but requires more ATP to fix one molecule of CO_2 than C_3 photosynthesis does. The NDH-1 complex accumulates in high amounts in bundle sheath cells of NADP-malic enzyme (NADP-ME)–type C_4 species and in mesophyll cells of NAD-ME-type C_4 species, where there is a strong need for ATP, and it has been assumed that NDH-1-mediated CEF supplies the extra ATP needed for C_4 photosynthesis (129).

4.5. The Role of NDH in Acclimation to the Environment

Although mutants lacking the NDH-1 complex have no phenotype under normal growth conditions, growth defects have been reported in response to different stress conditions, including high light (33), water deficiency (57), and low temperature (148). Such stress conditions are known to induce a high reducing state in the stromal pool that would favor the activity of CEF (114). Similar increases in CEF were observed in mutants affected in Calvin-Benson-cycle enzymes (72, 73), and it has been suggested that H_2O_2 produced in response to metabolic disorders or stress conditions may increase NDH-1-mediated CEF (20, 72, 114). Indeed, H_2O_2 mediates the induction of the NDH-1 complex (20) as well as the phosphorylation of the NdhF subunit (71). More recently, Strand et al. (126) showed that H_2O_2 directly and specifically activates the CEF pathway involving NDH-1. Therefore, the NDH-1-mediated CEF pathway may be activated under highly reducing conditions by the production of H_2O_2.

4.6. Hydrogen Photoproduction in Cyanobacteria and Microalgae

Some cyanobacterial and microalgal species are able to produce hydrogen in light thanks to a tight coupling between the photosynthetic electron transport chain and a hydrogenase (2, 81). The conversion of solar energy into molecular hydrogen by photosynthetic microorganisms, using water as an electron donor, is an important biotechnological issue, but production rates of wild-type strains need to be improved (34). NDH-1 and NDH-2 are associated with the process of hydrogen photoproduction in cyanobacteria and microalgae, respectively (6, 23, 24).

Synechocystis harbors a reversible [NiFe] hydrogenase that functions mainly as an uptake hydrogenase. The wild-type strain produces very little hydrogen in light, whereas mutant strains impaired in the NDH-1 complex show sustained hydrogen production (23). Gutekunst et al. (48) recently reported that the *Synechocystis* [NiFe] hydrogenase uses Fd as an electron donor. In this

context, the increased hydrogen production observed in the NDH mutant may be due to increased electron flow to the hydrogenase in the absence of NDH-1, with both enzymes using reduced Fd as an electron donor.

Microalgae such as *Chlamydomonas* spp. harbor an Fe-only hydrogenase using reduced Fd as an electron donor. One of the major limitations of hydrogen production by photosynthetic organisms is related to the high oxygen sensitivity of the Fe-only hydrogenase and to the fact that PSII produces molecular oxygen in light. By allowing the introduction of electrons stored as starch during oxygenic photosynthesis, the plastidial NDH-2 enables this limitation to be overcome. Based on a study of microRNA lines expressing reduced levels of CrNda2, Jans et al. (65) concluded that this enzyme is involved in the reduction of the PQ pool and in hydrogen photoproduction. Baltz et al. (65) further confirmed the involvement of CrNda2 in hydrogen production by overexpressing this enzyme and showing that CrNda2 supplies electrons to the indirect hydrogen production pathway, thereby demonstrating that nonphotochemical reduction of PQ is a limiting step in conditions where the stromal NAD(P)H pool is sufficiently reduced. Therefore, despite their completely different bioenergetic contexts, NDH-1 and NDH-2 have proven to be attractive targets for improving hydrogen production rates in cyanobacteria and microalgae, respectively.

4.7. The Role of NDH-2 in Prenylquinone and Vitamin K_1 Metabolism

A study of a mutant defective in AtNDC1, the unique *Arabidopsis* NDH-2 targeted to the chloroplast, showed that although this enzyme is able to reduce PQs, it is not involved in CEF or chlororespiration (108). Indeed, AtNDC1 is located in chloroplast lipid droplets (or plastoglobules), where it participates in prenylquinone metabolism and the α-tocopherol redox cycle (108). AtNC1 would be involved in the regeneration of oxidized α-tocopherol produced in response to high light by reducing α-tocopherol quinone to α-tocopherol quinol (109). A recent study showed that AtNDC1 and its *Synechocystis* ortholog ndbB are actually bifunctional oxidoreductases that are able to act on both prenyl naphtoquinones and prenyl benzoquinones and are involved in the penultimate step of vitamin K_1 (phylloquinone) synthesis (37).

5. NDH-1, NDH-2, AND THE EVOLUTION OF OXYGENIC PHOTOSYNTHESIS

Both NDH-1 and NDH-2 are structurally related to the machinery of respiratory electron transport. Even though NDH-1 and NADH dehydrogenases have common origins, they evolved differently and exhibit different activities.

5.1. Origin and Evolution of the Photosynthetic NDH-1

Even though they both contain a conserved L-shaped skeleton, photosynthetic NDH-1 and respiratory NADH dehydrogenase have different catalytic activities. How did these two enzymatic complexes diverge from their common origin? Analysis of sequence similarities between NADH dehydrogenase and membrane-bound [NiFe] hydrogenase (group 4) (17) led to the conclusion that these enzymes have a common ancestor (31, 43, 45) (**Figure 3**). The Ech hydrogenase of *Methanosarcina barkeri* belongs to group 4 and consists of six subunits (EchA–F) (76). EchA and EchB are membrane-embedded subunits that correspond to NdhF/NuoL and NdhA/NuoH, respectively. EchC–F correspond to NdhK/NuoB, NdhJ/NuoC, NdhH/NuoD, and NdhI/NuoI, respectively, which form the Q module in respiratory NADH dehydrogenase. In Ech hydrogenase, EchF accepts electrons from Fd to reduce protons, with the electron

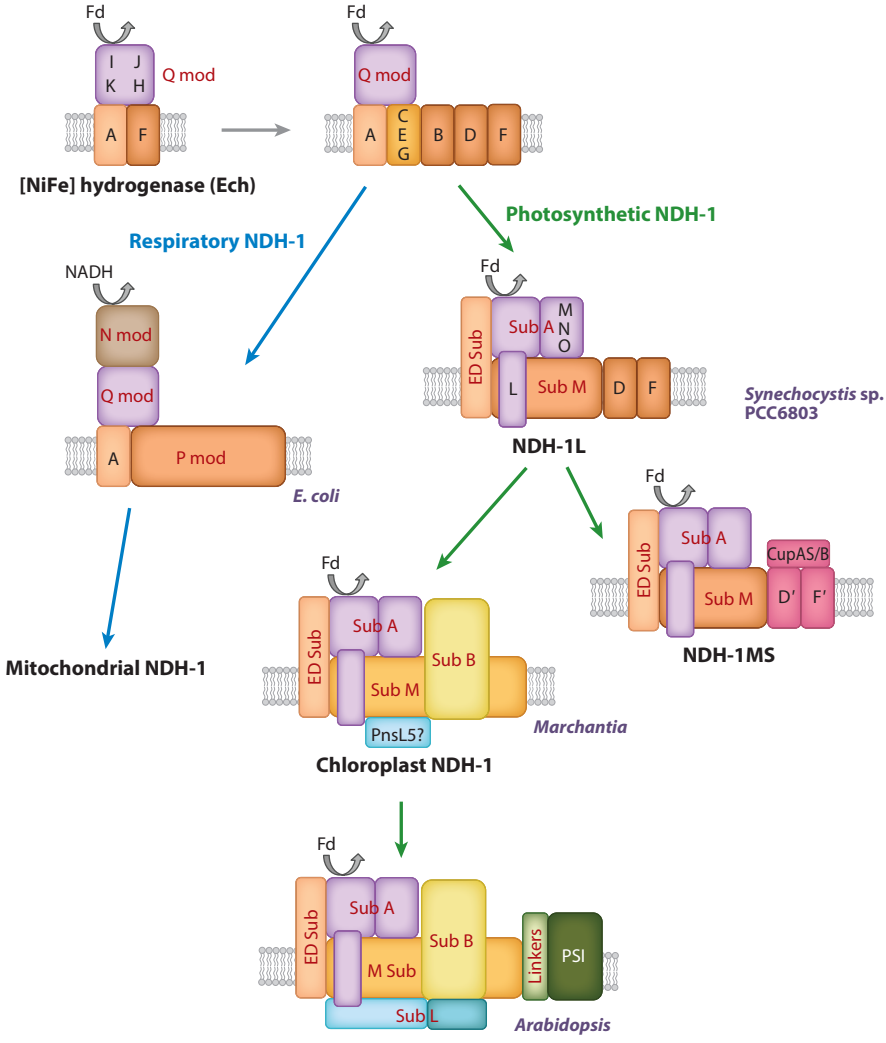

Figure 3

Evolution of NDH-1-related protein complexes. A group of proteins is thought to have originated from group 4 membrane-bound [NiFe] hydrogenases. Subunits are indicated by black letters on the basis of names in photosynthetic NDH-1; for example, A represents NdhA in photosynthetic NDH-1 but NuoH in *Escherichia coli* complex I and EchB in [NiFe] hydrogenase. The name of each subcomplex (sub) or module (mod) is indicated in red. Ech consists of four subunits that form the Q module along with two membrane subunits. The Q module mediates Fd-dependent quinone reduction. Triplication of F protein completed the M (membrane) subcomplex (P module plus A protein) as well as insertion of the C, E, and G proteins. In the evolution of respiratory NDH-1, the complex was equipped with the N module involved in NADH oxidation. In cyanobacteria, efficient Fd binding to the complex required subcomplex ED (electron donor), and NDH-1 was diversified into NDH-1L and NDH-1MS. Chloroplast NDH originated from NDH-1L. In *Marchantia polymorpha*, the complex is further equipped with subcomplex B. In *Arabidopsis*, subcomplex L (lumen) was completed, and NDH-1 interacts with PSI via linkers (Lhca5 and Lhca6) to form the supercomplex. Abbreviations: Fd, ferredoxin; PSI, photosystem I.

transport coupled with proton translocation through EchA. Because NdhI is homologous to EchF, NdhI potentially accepts electrons from Fd in photosynthetic NDH-1. However, the N module of respiratory NADH dehydrogenase is related to group 3 bidirectional cytoplasmic [NiFe] hydrogenases (31). In *E. coli*, formate hydrogenlyase core subunits forming the L-shaped skeleton interact with FdhF, which oxidizes formate (31). FdhF is homologous to NuoG, a subunit of the N module. The N module is further equipped with NuoE and NuoF to oxidize NADH. By contrast, photosynthetic NDH-1 retained the original electron input module, which accepts electrons from Fd. It seems likely that all of the complex I–related enzymes originated from a common ancestor with proton-transporting hydrogen:Fd oxidoreductase activity, which is most similar to group 4 membrane-bound [NiFe] hydrogenase, consisting of six subunits (43).

During the evolution of complex I–related enzymes, the membrane arm acquired more subunits, possibly via triplication of EchA/NdhF/NuoL, which resulted in the generation of NdhB/NuoN and NdhD/NuoM (31, 43). Additionally, NdhC/NuoA, NdhE/NuoK, and NdhG/NuoJ form another route of protons in the P module. NdhB, NdhD, and NdhF consist of 14 transmembrane helices and are homologous to each other and to the MrpA and MrpD subunits of the multiple resistant to pH (Mrp) Na^+/H^+ antiporter (79). NuoL is more closely related to MrpA, whereas NuoM and NuoN are more closely related to MrpD (78). Moparthi et al. (85) suggested that MrpA and MrpD have different functions in the Na^+/H^+ antiporter. Coupled with the movement of two electrons from the electron donor to quinone, four protons are pumped across the membrane. Whether this also applies to photosynthetic NDH-1 will need to be experimentally tested in the future.

5.2. Coevolution of NDH-1 and NDH-2 in the Green Lineage

In cyanobacteria, the NDH-1 complex is involved in numerous functions, including respiration, carbon concentration, and CEF around PSI, whereas the NDH-2 complex makes a limited contribution to respiration and may instead be involved in regulatory functions. As a result of the endosymbiotic event at the origin of chloroplasts, photosynthetic cells contain two respiratory electron transport chains, one mitochondrial and one chloroplastic. This functional redundancy may have resulted in a selection pressure that favored the specialization of plastid enzymatic complexes in the photosynthetic function. Despite this specialization, plastidial *ndh* genes, which represent approximately one-tenth of the 120 plastid-encoded genes, are highly conserved across all vascular plant divisions, indicating strong selection pressure (91).

However, plastidial *ndh* genes have been lost independently several times during the evolution of photosynthetic organisms. In parasitic organisms, *ndh* genes are the first to be lost during the transition from autotrophy to heterotrophy (125). Among microalgae, species from the red and green lineages, including Chlorophyceae, Ulvophyceae, and Trebouxiophyceae, have lost *ndh* genes, whereas they are present in early divergent Prasinophyceae and Nephroselmidophyceae (135). In angiosperms, the loss of *ndh* genes is a rare event that occurred in a clade of Geraniaceae (16) and in *Najas* species associated with the recolonization of aquatic environments (107). In gymnosperms, the loss of *ndh* genes is restricted to Gnetales and Pinaceae (18).

The moss *Physcomitrella patens* harbors a functional NDH-1 complex (3) and several plastid-targeted NDH-2s, including the cyanobacterial type and two from the NDB group (PpNDB1 and PpNDB2) that are phylogenetically close to CrNda2 (143). The plastid targeting of *Physcomitrella* and *Chlamydomonas* NDB proteins is due to the existence of an N-terminal extension that has been lost in vascular plants, likely owing to some functional redundancy between NDH-2 and the plastidial NDH-1 (143). As a result of this evolutionary process, *Arabidopsis* targets only one of its seven NDH-2s to chloroplasts, the cyanobacterial-type AtNDC (143). However, the function

of AtNDC is not redundant with NDH-1, as this plastoglobule-associated enzyme is involved in prenylquinone and vitamin K_1 metabolism (37, 108), which may explain why NDC is the only plastidial NDH-2 found in all photosynthetic organisms.

Therefore, although the NDH-1 complex is extremely well conserved in most land plants and some algae, it disappeared independently several times over the course of evolution. It will be of interest to determine whether the disappearance of the plastidial NDH-1 in some species is associated with the plastidial targeting of NDH-2s that may have functionally replaced NDH-1.

6. CONCLUDING REMARKS

The chloroplasts of both photosynthetic eukaryotes and cyanobacteria contain an NDH-1 complex that functions in close relationship with photosynthesis. Although our understanding of NDH-1 composition, biogenesis, and functioning has greatly improved over the last decade, revealing a high degree of complexity, its physiological function remains obscure. This may be due partly to its redundancy with other pathways (PGR5/PGRL1, NDH-2, flavodiiron proteins, etc.) and partly to the fact that, depending on the cellular context, NDH-1 and NDH-2 have developed specialized functions. Future work should clarify the regulatory roles of NDH-1 and NDH-2 in relation to the acclimation of plant photosynthesis to specific environments.

SUMMARY POINTS

1. Recent studies combining genetic and biochemical approaches have demonstrated a high degree of complexity of NDH-1s, which harbor at least 18 subunits in cyanobacteria and 30 in chloroplasts.
2. Cyanobacterial NDH-1s show a high degree of diversity: Four complexes (NDH-$1_{1–4}$) differing in the nature of their NdhD and NdhF subunits are specialized in different metabolic functions, including respiration (NDH-$1_{1,2}$), cyclic electron flow (CEF) (NDH-$1_{1–4}$), and CO_2-concentrating mechanisms (NDH-$1_{3,4}$).
3. The chloroplast NDH-1 complex forms a supercomplex with photosystem I and participates in one pathway of CEF; the other pathway involves PGR5/PGRL1. In organisms such as microalgae, which lack NDH-1, a plastidial NDH-2 is involved in CEF. These two pathways generate a component of the proton motive force that is used to produce extra ATP for CO_2 fixation or to trigger regulatory mechanisms of linear electron flow, such as nonphotochemical quenching or photosynthetic control.
4. Although NDH-1s were initially thought to use NAD(P)H as an electron donor, recent studies have shown that both chloroplast and cyanobacterial NDH-1s use reduced ferredoxin and should be considered ferredoxin-plastoquinone reductases rather than genuine NADH dehydrogenases.
5. NDH-1 and NDH-2 are important biotechnological targets for optimizing the hydrogen production abilities of cyanobacteria and microalgae, respectively.
6. Some plant and microalgal species have independently lost plastidial *ndh* genes and a functional NDH-1 during evolution, thus showing that NDH-1 can be dispensable.
7. The existence of conserved subunits and structural features (L shape) indicates that respiratory NADH dehydrogenase and plastidial NDH-1 most likely originated from a common ancestor, the [NiFe] hydrogenase Ech.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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