Running Title: Nomenclature for new subunits of chloroplast NDH

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Structure of the chloroplast NADH dehydrogenase-like complex: nomenclature for nuclear-encoded subunits

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Abbreviations: CET, cyclic electron transport; Fd, ferredoxin; LET, linear electron transport; NDH, NADH dehydrogenase-like complex; PQ, plastoquinone

Abstract

Chloroplast NADH dehydrogenase-like complex (NDH) was first discovered based on its similarity to complex I in respiratory electron transport, and is involved in electron transport from photoproduced stromal reductants such as NADPH and ferredoxin to the intersystem plastoqunone pool. However, a recent study suggested that it is a ferredoxin-dependent plastoquinone reductase rather than an NAD(P)H dehydrogenase. Furthermore, recent advances in subunit analysis of the NDH complex have revealed the presence of a novel hydrophilic subcomplex on the stromal side of the thylakoid membrane, as well as an unexpected lumenal subcomplex. This review discusses these new studies on the structure of the NDH complex, and proposes a unified nomenclature for newly discovered NDH subunits.

Introduction

Light reactions of photosynthesis comprise the electron transport in the thylakoid membrane. Electrons excised from water in photosystem (PS) II are transported to PSI through the cytochrome (Cyt) b_6f complex and eventually produce NADPH. Protons are translocated from the stroma to the lumen across the thylakoid membrane in the steps coupled to the electron transport and the resulting ΔpH, as well as ΔpH generated by lumenal water oxidation in PSII, is utilized to produce ATP. In addition to this linear electron transport (LET) from water to $NADP^+$, cyclic electron transport (CET) around PSI recycles electrons from ferredoxin (Fd) or NADPH photoreduced by PSI to the plastoquinone (PQ) pool, which mediates the electron transport between PSII and Cyt b_6f in the thylakoid membrane (Shikanai 2007). Consequently, ΔpH is formed without production of NADPH in PSI CET.

In PSI CET, it has been believed that electrons are recycled to PQ via either an antimycin A-sensitive pathway (Tagawa et al. 1963, Munekage et al. 2002) or an NADH dehydrogenase-like complex (NDH)-dependent pathway (Burrows et al. 1998, Kofer et al. 1998, Shikanai et al. 1998). The antimycin A-sensitive pathway was discovered by Arnon and coworkers (Tagawa et al. 1963) and is often called the Fd-dependent pathway. Since a recent report strongly suggested that NDH also accepts electrons from Fd (Yamamoto et al. 2011), we here do not use the term "Fd-dependent pathway" to avoid confusion and propose that this term should no longer be used.

The physiological role of CET has been suggested to protect PSII under strong light

via ΔpH-dependent thermal dissipation in PSII as well as an ATP generator in photosynthesis (Heber and Walker 1992). More recent views of the physiological roles of PSI CET have been described elsewhere (Endo et al. 2008, Miyake 2010, Johnson 2011).

More than half a century has passed since Arnon and coworkers discovered the "cyclic photophosphorylation" sensitive to antimycin A, an inhibitor of the Cyt b/c_1 complex in mitochondria (Tagawa et al. 1963). However, the route taken by electrons in this pathway still remains unclear. Genetic studies using Arabidopsis have clarified two proteins, the PGR5 and PGRL1 (PGR5-LIKE PHOTOSYNTHESIS PHENOTYPE 1) proteins, a deficiency of which results in the total inactivation of antimycin A-sensitive Fd-dependent PQ reduction activity (Munekage et al. 2002, DalCorso et al. 2008), although the exact molecular function of these proteins is unclear (Nandha et al. 2007). The recent discovery of the PSI-LHCI-LHCII-FNR-Cyt $b_6 f$-PGRL1 super-complex suggests direct electron donation from Fd to the Cyt $b_6 f$ complex in *Chlamydomonas reinhardtii* (Iwai et al. 2010). In Arabidopsis, however, CET is independent of the state transition, in which LHCII is associated with PSI, and such a large protein complex has not been discovered.

Quantitative contributions of NDH and PGR5/L-1 to total CET is not clearly demonstrated due to the rack of the kinetics data. However, we believe that it is reasonable to assume that NDH and PGR5/L-1 function in cyclic electron transport because both are essential for electron donation to the photosynthetic plastoquinone pool from reduced ferredoxin, the end product of photosynthetic electron transport

(Munekage et al. 2004). And it is also clear that NDH- and PGR5-dependent electron flows are essential to support photosynthetic growth of plants because lack of both pathways results in very severer growth inhibition (Munekage et al. 2004).

In this review, we focus on the chloroplast NDH complex. The NDH-dependent pathway was first experimentally demonstrated in cyanobacteria, the ancestor of chloroplasts (Mi et al. 1994, 1995).   In flowering plants, chloroplast NDH is believed to mediate the alternative CET pathway, although exceptions can be found: Some plants, including black pine, have lost the chloroplast NDH during evolution (Wakasugi et al. 1994), and a similar loss has also occurred in green algae, including Chlamydomonas. Extensive mutant studies have demonstrated that NDH does not function to generate pH gradient to trigger thermal dissipation in PSII, and the defective mutants of NDH do not show any clear phenotype when grown in controlled environments (Burrows et al. 1998, Shikanai et al. 1998). Nevertheless, the physiological relevance of NDH-mediated electron flow in chloroplasts of C3 plants, particularly under stress conditions, has been frequently demonstrated (Endo et al. 1999, Horváth et al. 2000, Hashimoto et al. 2003, Munekage et al. 2004, Li et al. 2004, Wang et al. 2006).

The ATP supply by CET has been reported to be very important in C4 plants, which have higher ATP requirements than C3 plants (Heber and Walker 1992), and evidence for NDH in such a role in C4 plants was provided by comparative expression analyses of an NDH subunit and PGR5 in the mesophyll and bundle sheath cells of several C4 plants (Takabayashi et al. 2005). Based on mathematical modeling of C4 photosynthesis, Laisk and Edwards (2000, 2009) proposed that electrons actually do not

cycle around PSI in the bundle sheath, but enter via NDH into the PQ pool right after passing through PSI only once.

Recent research on NDH complexes in flowering plant, such as tobacco (*Nicotiana tabacum*), Arabidopsis and maize (*Zea mays*), has revealed the presence of several novel subunits and entire subcomplexes on both the stromal and lumenal sides of the thylakoid membrane (for more detailed information on the structure of NDH, see Peng et al. 2011a). Moreover, a supercomplex structure containing both PSI and NDH complexes has been demonstrated (Peng et al. 2008), but the amount of these supercomplexes is rather low since the stoichiometry of the NDH complex is about 1% of the PSI complex. Several groups have independently identified novel NDH subunits with the consequence that some proteins have two names, and even more problematically, the same names were assigned to different NDH subunits. In order to solve the current confusion, we here provide a brief overview of the present understanding of the structure of the chloroplast NDH complex and propose to unify the nomenclature for NDH subunits in flowering plant chloroplasts. New names of NDH subunits proposed below are listed in Table 1. We also propose the renaming of mutants accordingly.

"Photosynthetic NDH" and "respiratory NDH"

Although the chloroplast *ndh* genes were first discovered on sequenced plastid genomes of tobacco and a liverwort (*Marchantia polymorpha)* based on the similarity with subunit genes of mitochondrial complex I, it was later shown to be more similar to

bacterial NDH-1, especially to cyanobacterial NDH-1.   The bacterial respiratory

Complex I (NDH-1) is generally composed of 14 subunits (for a review, see Friedrich

1998).   An exception is observed in cyanobacteria that encode proteins corresponding

to only 11 subunits (NdhA-NdhK) of non-phototrophic bacterial NDH-1. The missing

three subunits correspond to 51, 24 and 75 kDa proteins in *Bos taurus* and NuoE, F and

G of *E. coli* (Friedrich and Scheide 2000).   The 51 kDa (NuoF) subunit contains FMN

and may have an NADH-binding domain.   Thus, these three subunits have been

referred to as the NADH-binding subcomplex (Friedrich 1998). The plastid genomes of

flowering plants also have 11 genes (*ndhA-ndhK*) which are conserved in cyanobacterial

genomes. Even after the Arabidopsis nuclear genome was completely sequenced, no

candidates were found as orthologs of the NADH-binding subunits of chloroplast NDH,

except for those of mitochondrial complex I (Grohmann et al. 1996).   This suggests

that chloroplast and cyanobacterial NDH has an electron donor-binding subcomplex

that is unique to oxygenic photosynthetic organisms.

In *Synechocystis* PCC6803, NDH subunits form three types of complexes with

different subunit compositions (Zhang et al. 2004).   NDH-1L is required for

heterotrophic growth, probably via respiration and CET, while NDH-1M and NDH-1S

form the NDH-1MS complex that functions in CO_2 concentration (Zhang et al. 2004,

for a recent review, see Battchikova et al. 2011).   Based on the similarity of NdhD and

NdhF, the chloroplast NDH is believed to be related to cyanobacterial NDH-1L, and this

is consistent with the idea that chloroplast NDH is involved in PSI CET and respiration

(Shikanai 2007). Thus, the NDH complex in chloroplasts and cyanobacteria can be

referred to as "photosynthetic NDH", which has a unique structure and function distinguishable from "respiratory NDH".   To avoid confusion, it should be noted that cyanobacterial NDH is involved in respiration as well as in photosynthesis.   It is conceivable that chloroplast NDH may also be involved in the electron transport from stromal reductants to oxygen in chlororesipiration.

Discovery of subunits specific to photosynthetic NDH

Since photosynthetic NDH apparently lacks subunits involved in electron input, priority was given to the identification of subunits specific to photosynthetic NDH.   The first successful approach was purification of the hydrophilic part of NDH from tobacco chloroplasts via the His-tag added to NdhH by a technique of plastid transformation (Rumeau et al. 2005).   Through combination with reverse genetics using Arabidopsis, the nuclear-encoded NdhM-NdhO subunits were shown to be genuine NDH subunits. The homologs of NdhM-NdhO have been discovered in cyanobacterial NDH-1, but not in respiratory complex I, suggesting that NdhM-NdhO are NDH subunits specific to photosynthetic NDH (Prommeenate et al. 2004, Battchikova et al. 2005).

Another subunit specific to photosynthetic NDH is NdhL. The *ndhL* gene was originally discovered in cyanobacteria based on its response to a low CO_2 concentration (Ogawa 1992), and subsequently the NdhL protein was discovered by a proteomics approach (Battchikova et al. 2005). Its ortholog is encoded in the Arabidopsis nuclear genome, and genetic and biochemical studies have shown that NdhL is a subunit of chloroplast NDH (Shimizu et al. 2008).

Subcomplex structure of flowering plant NDH with specific subunits

After the nuclear-encoded proteins NdhL-NdhO were established as chloroplast NDH subunits, several novel subunits have since been discovered.   From the viewpoint of evolution, the subunits of photosynthetic NDH can be categorized into two distinct groups: evolutionarily old, basic subunits common in cyanobacteria and eukaryotic phototrophs, and new subunits that are unique to flowering plants.   Some homologs may be encoded in the genome of a moss (*Physcomitrella patens*), but their function is still unclear.   The old subunits form the L-shape complex that is common to photosynthetic and respiratory NDH complexes (Fig. 1). In contrast, the new subunits seem to comprise unique subcomplexes that are not found in cyanobacteria.

Several candidates for novel NDH subunits were identified in a proteome study that focused on proteins that specifically accumulated in chloroplasts of maize bundle sheath cells (Majeran et al. 2008).   Sirpiö et al. (2009a) and Takabayashi et al. (2009) independently showed that some of these proteins are genuine subunits of the NDH complex in Arabidopsis, and may comprise a second hydrophilic arm on the stromal surface of NDH.   Based on the stability of proteins in the various mutant backgrounds, these hydrophilic subunits together with several subunits with membrane-spanning domains were proposed to form the part called subcomplex B to distinguish it from the hydrophilic arm conserved in cyanobacterial NDH-1 (subcomplex A) (Peng et al. 2009). Another group that is probably unique to flowering plants is localized on the lumenal surface of the thylakoid membrane (Ishihara et al. 2007, Majeran et al. 2008, Peng et al.

2009, Suorsa et al. 2010, Yabuta et al. 2010). In summary, the chloroplast NDH in flowering plants is postulated to be comprised of four distinct subcomplexes: A, B, membrane, and lumen-localized subcomplexes.

Membrane and "A" subcomplexes

All of the NADH dehydrogenase-related complexes are believed to form the L-shape structure (Arteni et al. 2006, Efremov et al. 2010). Seven plastid genes (*ndhA-ndhG*) encode subunits of the membrane subcomplex. Four additional plastid genes (*ndhH-ndhK*) encode the basal part of subcomplex A, which probably forms the skeleton of the L-shape structure along with the membrane subcomplex (Battchikova et al. 2011).   Since 11 subunits are highly conserved in bacterial NDH-1, the 3D structure of which was recently resolved (Efremov et al. 2010), the membrane subcomplex is likely to pump protons across the thylakoid membrane coupled with the movement of electrons in subcomplex A of photosynthetic NDH. The dependence of the accumulation of nuclear-encoded NdhM-NdhO on that of NdhH-NdhL, and vice versa, provided evidence for the categorization of the NdhM-NdhO subunits into subcomplex A (Peng et al. 2009). In cyanobacteria, NdhM-NdhO were observed by electron microscopy to be attached to the membrane subcomplex but not to the hydrophilic arm corresponding to NdhH-NdhK (Birungi et al. 2010).   The structural model of the complex summarizes the interaction among subunits based on their stability in the various mutant backgrounds and does not necessarily provide information on the actual position of each subunit in the complex (Peng et al. 2009).

11

In contrast to hydrophilic NdhM-NdhO, NdhL contains three transmembrane domains in Arabidopsis (Shimizu and Shikanai 2007). NdhL is specifically required for the accumulation of subcomplex A, and vice versa , suggesting that NdhL is a component of subcomplex A (Peng et al. 2009). Consistently, the accumulation of NdhL was affected in the *crr6* and *crr7* mutants, both of which are defective in the assembly of subcomplex A in the stroma (Peng et al. 2010).

Subcomplex B

Novel nuclear-encoded subunits have been proposed to form a new subcomplex called subcomplex B, which is unique to flowering plants (Peng et al. 2009). When one subunit in this group was missing, all other subunits in the same group disappeared as well, and the level of subcomplex A subunits decreased to 10% of that in the wild type. This is contrasting to the fact that although a deficiency of any subunit in subcomplex A destabilizes all other subunits in the same subcomplex, it does not affect other parts of the NDH-PSI supercomplex (Ishida et al. 2009, Peng et al. 2009, Sirpiö et al. 2009a, Takabayashi et al. 2009).   Furthermore, while absence of the lumen subcomplex (see below) completely destabilizes subcomplex A, the level of subcomplex B subunits falls to 25-50% of that in the wild type (Peng et al. 2009). All of these results suggest that subcomplex B forms a second hydrophilic arm that extends to the stroma, and attaches to the membrane subcomplex by two transmembrane proteins, NDF6 (Ishikawa et al. 2008) and NDH18 (Peng et al. 2009).

Some subunits in subcomplex B were discovered independently by two groups, and

both-names are used in the literature: NDH48/NDF1 and NDH45/NDF2. We propose to rename the subunits in subcomplex B using serial numbers based on the order of their discovery.   Thus, NDH48/NDF1, NDH45/NDF2, NDF4, NDF6 and NDH18 should be called PnsB1, 2, 3, 4 and 5, respectively, where "PnsB" represents Photosynthetic NDH subcomplex B. Proposed names are listed in Table 1.

Lumenal subcomplex L

Recent studies on the function of homologs of oxygen-evolving complex (OEC) proteins of photosystem II unexpectedly revealed that several OEC homologs function in the chloroplast NDH complex (for a review, see Ifuku et al. 2010).   To date, one PsbP homolog, a PsbP-like protein 2 (PPL2), and three PsbQ homologs have been shown to be required for NDH activity (Ishihara et al. 2007, Suorsa et al. 2010, Yabuta et al. 2010). Proteomic and *in silico* analyses have also supported the notion that these OEC homologs are subunits of the NDH complex (Majeran et al. 2008, Peng et al. 2009, Suorsa et al. 2009, Takabayashi et al. 2009). The three PsbQ homologs were given different names by different groups (Peng et al. 2009, Suorsa et al. 2010, Yabuta et al. 2010). To avoid confusion, we propose that the subunits on the lumenal side of the NDH complex should be called PnsL1-5 (Photosynthetic NDH subunit of the lumenal location). Accordingly, the PsbP-like protein 2 (PPL2) should be called PnsL1. The two PsbQ homologs (PsbQ-like protein) should be named PnsL2 and 3 in the order of their chromosome positions: the At1g14150 protein should be PnsL2, and the At3g01440 protein should be PnsL3. For the other PsbQ homolog PQL3 (At2g01918, Yabuta et al.

2010), its cellular localization is not clear at present, although it has been shown to be required for the stable accumulation and activity of NDH. Therefore, we refrain here from naming this protein as a lumenal subunit of NDH.

Another group of thylakoid lumenal proteins associated with the chloroplast NDH are immunophilins that belong to the family of peptidyl-prolyl *cis-trans* isomerase (Majeran et al. 2008). Two distinct types of immunophilin, FKBP16-2 and CYP20-2, have been found in the NDH complex (Peng et al. 2009, Sirpiö et al. 2009b). Further biochemical analyses suggest that PnsL1 (PPL2), PnsL2 (At1g14150), FKBP16-2, and CYP20-2 form lumenal subcomplex L (large) that may be connected to stromal subcomplex A, possibly via NdhL transmembrane domains (Peng et al. 2009, Sirpiö et al. 2009b, Suorsa et al. 2010, Yabuta et al. 2010). Accordingly, the Ndh subunits FKBP16-2 and CYP20-2 should be called PnsL4 and PnsL5, respectively. PsnL3 (At3g01440) does not seem to belong to lumenal subcomplex L but is rather associated with subcomplex B via PnsB4 (Suorsa et al. 2010, Yabuta et al. 2010) .

NDH-PSI supercomplex

Another striking feature of chloroplast NDH in flowering plants is its association with at least two copies of PSI to form the NDH-PSI supercomplex (Peng et al. 2008). This issue was extensively discussed in a recent review (Peng et al. 2011a). Two minor light-harvesting complex I proteins (Lhca5 and Lhca6) play a critical role in the formation of this supercomplex (Peng et al. 2009, Peng and Shikanai 2011), which in turn is required for stabilizing NDH, especially under high light conditions (Peng and

Shikanai 2011). It is still unclear why monomeric NDH without PSI is unstable in flowering plants.

Electron donor subunits

Photosynthetic NDH lacks subunits that function in NADH binding and the electron donor to the complex has been unclear. Furthermore, Fd but not NAD(P)H donates electrons to chloroplast NDH in isolated thylakoids, which suggests that NDH accepts electrons from Fd (Endo et al. 1997, Munekage et al. 2004). Recently, the discovery of CRR31 protein was reported, which is required for the high-affinity binding of Fd to NDH (Yamamoto et al. 2011). The C-terminal region of CRR31 is predicted to form the Src homology 3 (SH3) domain-like structure, and this structure is similar to that of PsaE. PsaE is a peripheral subunit of PSI and its SH3 domain-like structure forms the binding site of PSI to Fd with PsaC and PsaD.   Consistent with this fact, CRR31 is required for the high-affinity binding of Fd to NDH in the *in vitro* Fd-dependent PQ reduction assay system.   In such an assay with ruptured chloroplasts, PQ is reduced by exogenously added Fd, which in turn is photo-reduced by PSI via membrane-bound FNR, and the process is monitored as an increase in the apparent F_o level fluorescence emitted from PSII (Mills et al. 1979, Endo et al. 1997, Munekage et al. 2004). These results strongly suggest that CRR31 forms the Fd-binding site of NDH and NDH is Fd-dependent PQ reductase (FQR) rather than NAD(P)H dehydrogenase. This activity is resistant to antimycin A and thus independent of PGR5-dependent FQR activity. Based on these experimental results, Yamamoto et al. (2011) proposed that NDH should be renamed

NADH dehydrogenase-like complex.

CRR31 was originally discovered in proteomic studies that focused on the NDH-PSI supercomplex (Yamamoto et al. 2011). CRR31 is likely to interact with J protein (CRRJ) and J-like protein (CRRL), both of which have a transmembrane domain, and probably further with subcomplex A of NDH (Yamamoto et al. 2011). The putative electron donor-binding NDH subcomplex including CRR31, CRRJ and CRRL, accumulates partly independently of subcomplex A (Yamamoto et al. 2011). We consider this to be the fifth NDH subcomplex, including still unknown Fd-oxidizing subunits, and propose that its subunits should be renamed NdhS (CRR31), NdhT (CRRJ) and NdhU (CRRL). The electron-donor binding subunit of NDH is extremely fragile in blue-native gel, and it is unclear how electrons are transferred from Fd to electron carriers present in subcomplex A. The C-terminal region of NdhS (CRR31) is conserved in cyanobacteria, and this electron input module of NDH may be conserved in photosynthetic NDH.

Other NDH-related proteins

In addition to genuine subunits of the NDH complex, some protein factors that are essential for the accumulation of NDH have been isolated by forward genetics. CRR3 is a small protein with a transmembrane domain and is a candidate NDH subunit (Muraoka et al. 2006). However, CRR3 has not been identified in proteomics approaches and it is still not clear whether it is really a subunit of NDH.

A group of genes are required for the expression of plastid *ndh* genes. They are

involved in transcription and RNA maturation in plastids. Recent progress in this field has been reviewed elsewhere (Suorsa et al. 2009, Peng et al. 2011a). It is still unclear why so many factors are required for the regulation of plastid *ndh* genes.

Another group of genes are required for assembly of the chloroplast NDH complex. CRR6 and CRR7 are stromal proteins and their defects lead to the absence of subcomplex A (Peng et al. 2010). CRR6 is required for the formation of an assembly intermediate of subcomplex A in the stroma, while CRR7 is probably involved in a later step such as the insertion of subcomplex A into the thylakoid NDH intermediate. While NDF5 shows similarity to PnsB2 (NDF2/NDH45), they have distinct functions, since NDF5 was not found in mature NDH complex, although it is essential for assembly of the NDH complex in the thylakoid membrane (Ishida et al. 2009). CRR1 shows weak similarity to dihydrodipicolinate reductase involved in lysine biosynthesis, but its defects lead to the specific loss of chloroplast NDH (Shimizu and Shikanai 2007). CRR1 is also likely to be involved in assembly of the NDH complex in the stroma (Peng et al. unpublished). The *CRR27* gene encodes a chaperonin subunit, Cpn60β4, which has an extremely low expression level compared to that of other chaperonins (Peng et al. 2011b). This unusual chaperonin subunit with a unique C-terminal extension has been shown to be a subunit of the Cpn60 complex and this specific Cpn60 complex including Cpn60β4 is specifically required for the folding of NdhH.

Forward genetics approaches originally sought to identify missing subunits involved in electron donor binding, but unexpectedly resulted in the identification of a

large number of factors involved in the assembly of the NDH complex. Chloroplast NDH is dispensable under growth chamber conditions and mutations are unlikely to have any severe secondary effects on chloroplast function. Based on innovative developments in the recent proteomics approach, it is not very difficult to analyze the assembly intermediates of chloroplast NDH, the level of which is only 1% of that of PSI. Chloroplast NDH may become a model, on the side of photosystem II, for studying the assembly of the protein megacomplex in the thylakoid membrane.

Perspectives

Recent extensive studies have identified new subunits of NDH, and a unique subcomplex structure has been proposed (Fig. 2). The identification of NdhS (CRR31) strongly suggests that NDH accepts electrons from Fd.　Although the route that electrons follow from Fd to PQ is still unclear, chloroplast NDH is no longer "the complex of enigma", at least as far as its structure is concerned.　Despite the high similarity in the L-shape backbone, including NdhL-NdhO, between cyanobacterial NDH-1L and chloroplast NDH, the latter is equipped with two specific subcomplexes and further associates with PSI in flowering plants (Peng et al. 2009).　Future studies will be needed to clarify the point in evolution at which the structure of chloroplast NDH changed drastically in land plants.　In addition, we must determine why this change was necessary.　Molecular genetics has demonstrated the physiological relevance of chloroplastic NDH in flowering plants. Compared to the PGR5/PGRL1-dependent, antimycin A-sensitive PSI CET, the rate of NDH-dependent

PSI CET was estimated to be very low (Munekage et al. 2004, Okegawa et al. 2008). NDH may help to protect the photosynthetic machinery from oxidative damage in chloroplasts rather than contributing to ATP synthesis during photosynthesis, whereas it is still unclear how NDH alleviates oxidative stress. Our understanding of the physiological function(s) of NDH and CET in general are still rather circumstantial and superficial. For proper understanding, we have to know the e^-/H^+ and H^+/ATP ratios resulted from each type of CET (for example, see Steigmiller et al. 2008). Moreover, careful kinetic analyses are required to quantitate the allocation of light energy absorbed in PSII and PSI to LET, CETs and other alternative electron transport routes (Huang et al, 2010, 2011; Miyake 2010, Laisk et al. 2007, 2010).

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Table 1 List of nuclear-encoded subunits and protein factors of chloroplast NDH

complex

| Subcomplex	Original name	Proposed name	AGI code	motif	reference
A	NdhL/CRR23	NdhL	At1g70760	transmembrane	Shimizu et al. (2008)
	NdhM	NdhM	At4g37925		Rumeau et al. (2005)
	NdhN	NdhN	At5g58260		Rumeau et al. (2005)
	NdhO	NdhO	At1g74880		Rumeau et al. (2005)
B	NDH48/NDF1	PnsB1	At1g15980		Majeran et al. (2008); Sirpiö et al. (2009a); Takabayashi et al. (2009)
	NDH45/NDF2	PnsB2	At1g64770		Majeran et al. (2008); Sirpiö et al. (2009a); Takabayashi et al. (2009)
	NDF4	PnsB3	At3g16250	Fe-S center	Majeran et al. (2008); Takabayashi et al. (2009)
	NDF6	PnsB4	At1g18730	transmembrane	Ishikawa et al. (2008)
	NDH18	PnsB5	At5g43750	transmembrane	Peng et al. (2009)
Lumenal	PPL2	PnsL1	At2g39470	PsbP family	Ishihara et al. (2007)
	PQL1/PQL2/PsbQ-F1	PnsL2	At1g14150	PsbQ family	Peng et al. (2009) Suorsa et al. (2010) Yabuta et al. (2010)
	PQL2/PQL1/PsbQ-F2	PnsL3	At3g01440	PsbQ family	Majeran et al. (2008) Peng et al. (2009) Suorsa et al. (2010) Yabuta et al. (2010)
	FKB16-2	PnsL4	At4g39710		Majeran et al. (2008);

|					Peng et al. (2009)
	AtCYP20-2	PnsL5	At5g13120		Majeran et al. (2008); Sirpiö et al. (2009b)
Linkers	Lhca5		At1g45474		Peng et al. (2009)
	Lhca6		At1g19150		Peng et al. (2009) Peng and Shikanai (2011)
Electron donor binding	CRR31	NdhS	At4g23890	SH3-like fold	Yamamoto et al. (2011)
	CRRJ	NdhT	At4g09350	J-domain, transmembrane	Yamamoto et al. (2011)
	CRRL	NdhU	At5g21430	J-like domain, transmembrane	Yamamoto et al. (2011)
Others	PQL3		At2g01918	PsbQ family	Yabuta et al. (2010)
	CRR1		At5g52100	DDPR-like	Shimizu and Shikanai (2007)
	NDF5		At1g55370PnsB2 homolog	PnsB2 homolog	Ishida et al. (2009)
	CRR6		At2g47910		Peng et al. (2010b)
	CRR7		At5g39210		Peng et al. (2010b)
	CRR3		At2g01590	transmembrane	Muraoka et al. (2006)

Legends for Figures

Fig. 1 Basic subcomplex compositions of the NDH complex in heterotrophic bacteria and photosynthetic organisms. UQ: ubiquinone in cell membranes, PQ: plastoquinone in thylakoid membranes.   Cyanobacterial NDH-1 is composed of subunits common to heterotrophic bacteria (NdhA-K, shown in red) and newly found subunits common to chloroplastic NDH complex (NdhL-O, shown in black).

Fig. 2 Subcomplex composition of the NDH complex in land plant chloroplasts. The NDH complex is composed of plastid-encoded subunits (red) and nuclear-encoded subunits (black).   NDH interacts with at least two copies of PSI via Lhca5 and Lhca6. Although NdhL has transmembrane domains, we categorize this polypeptide as a member of subcomplex A based on the intrinsic association of NdhL with other members of subcomplex A.   PsnL3 is closely associated with subcomplex B, and probably does not belong to lumenal subcomplex L (light green) which is composed of PsnL1, 2, 4 and 5.

Fig. 1
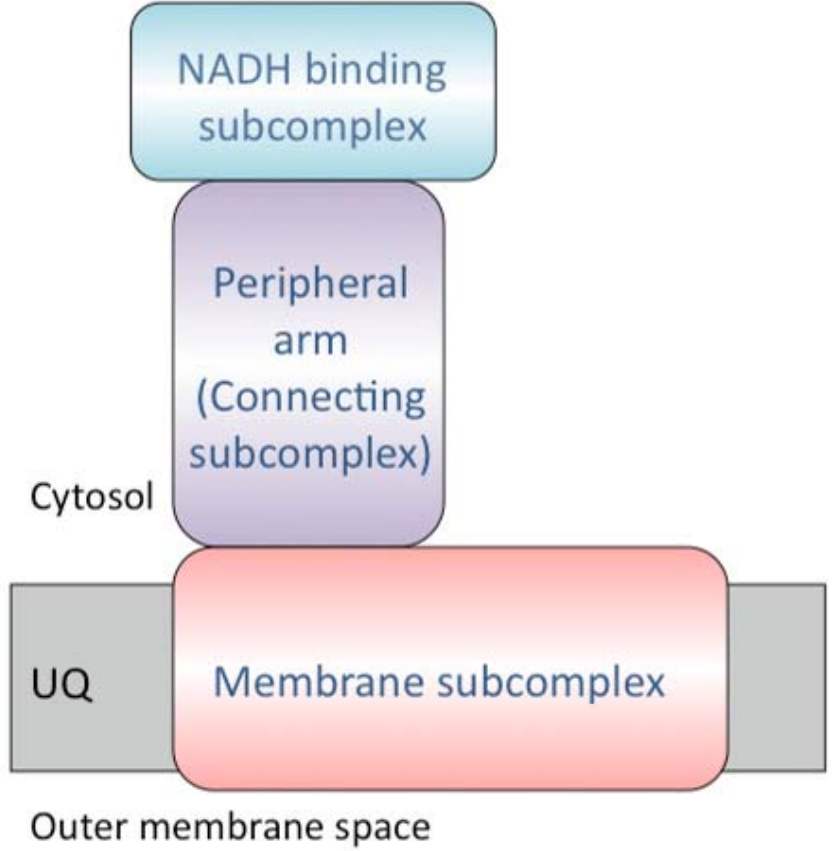
NDH-1 in heterotrophic bacteria
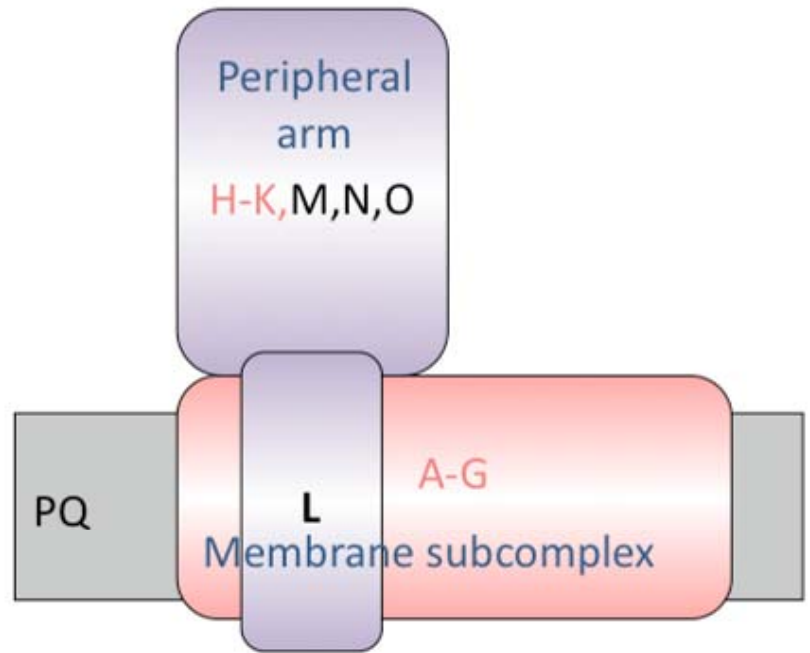
Backbone structure of NDH complex
Common in cyanobacteria and chloroplasts

Fig. 2