特约综述 Invited Review

叶绿体NAD(P)H脱氢酶(NDH)复合体的研究进展

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摘要: 高等植物叶绿体类囊体膜上的NAD(P)H脱氢酶(NDH)复合体是一个由多亚基组成的大复合体。它参与了围绕光系统I (PSI)的循环电子途径(CEF)和叶绿体的呼吸, 为CO_2同化提供额外ΔpH和ATP, 并在胁迫条件下缓解基质过还原和氧化胁迫。近年来高等植物的叶绿体NDH复合体的研究有了很大进展, 主要是发现了很多NDH新亚基、调控叶绿体编码的*ndh*基因的剪切和编辑的蛋白以及影响NDH组装和稳定的蛋白, 同时, 对NDH在胁迫条件下的生理功能也有了进一步的认识。本综述主要总结了NDH复合体的功能, 亚基组成及调控等方面的研究进展。

关键词: NAD(P)H脱氢酶复合体; 循环电子途径; 亚基; 光合作用

The Research Progress of Chloroplast NAD(P)H Dehydrogenase (NDH) Complex

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Abstract: The higher plant chloroplast NAD(P)H dehydrogenase (NDH) complex is a big multiple-subunit enzyme complex in thylakoid mambranes. NDH participates in the photosystem I (PSI) cyclic electron flow (CEF) and chlororespiration, NDH-dependent CEF provides extra ΔpH and ATP for the CO_2 assimilation, and alleviates the oxidative stress caused by stromal over-reduction under stress conditions. Recently the great progress of research on higher plant chloroplast NDH complex has been made. Many novel subunits, proteins involved in splicing and editing of chloroplast-encoded *ndh* genes and the proteins essential for assembly or stabilization of the NDH complex were found. In addition, we got a well understanding of the physiological function of the NDH under stress. In this review, we summarize the research progress of the function, the subunit composition and the regulation of the NDH complex.

Key words: NAD(P)H dehydrogenase complex; cyclic electron transport; subunit; photosynthesis

光合作用中的光反应在放氧光合生物的类囊体膜上发生, 其中的电子传递包括两部分, 线性电子传递和围绕光系统I的循环电子传递。在线性电子传递中, PSII反应中心被光能激发后, 引起电荷分离, 从水分子中夺取电子, 同时放出氧气。来自水分子的电子经过一系列电子递体的传递, 最终使$NADP^+$还原, 并耦联产生跨类囊体膜质子梯度(ΔpH), 驱动ATP合酶合成ATP, 用于CO_2同化反应。这个反应需要两个光化学反应中心驱动, 光系统II (PSII)和光系统I (PSI)。与这条电子传递链不同, 类囊体膜中还存在另外一种电子传递链, 只依赖PSI的光化学反应(Bendall和Manasse 1995)的循环电子传递链。电子从NADPH或者铁氧还蛋白(Fd)重新传递到质体醌(PQ)。围绕PSI的循环电子传递只产生用于合成ATP的ΔpH, 而不积累NADPH。对于循环电子传递的存在意义, 有过长期的争议。最近几年中, 随着循环电子传递研究的深入, 逐渐认识到循环电子传递在高等植物中起到重要和广泛的作用。NDH复合体也是叶绿体呼吸电子传递的组成部分, 因而作用光关闭后的叶绿素荧光上升后的下降阶段涉及与叶绿体呼吸有关的PTOX对PQ的再氧化(Feild等1998; Joet等2002; Martin等2004)。

在高等植物中, 已经鉴定到两条部分冗余的循环电子传递途径(Munekage等2004) (图1)。其中

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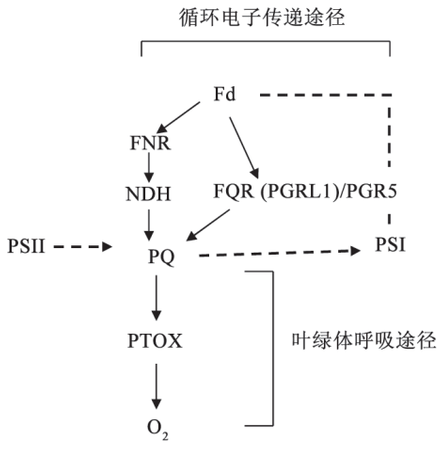

图1 围绕光系统I的循环电子传递和叶绿体呼吸途径
Fig.1 Cyclic electron flow around photosystem I and chlororespiration

根据米华玲(2003)文献修改。PSII: 光系统II; PSI: 光系统I; PQ: 质醌; NDH: NAD(P)H-脱氢酶; Fd: 铁氧还蛋白; FQR: 铁氧还蛋白-质醌还原酶; FNR: 铁氧还蛋白-$NADP^+$氧化还原酶; PTOX: 质体末端氧化酶; PGR5: 质子梯度调节蛋白; PGRL1: 质子梯度调节类似蛋白。

在C_3植物中主要的途径是对抗霉素A敏感的、由PGRL1/PGR5介导的循环电子传递途径。由于围绕PSI的循环电子传递途径产生跨类囊体膜ΔpH, 由此可以诱导非光化学猝灭(NPQ)的产生(Heber和Walker 1992)。通过筛选拟南芥的NPQ缺陷突变体, Munekage等(2002)发现了*pgr5*突变体, *pgr5*突变体的循环电子传递能力严重受抑制。Dalcorso等(2008)通过对拟南芥*pgrl1*突变体的研究进一步发现, PGRL1可能与PGR5相互作用, 共同调节围绕PSI的循环电子传递。最近Hertle等(2013)进一步指出PGRL1作为铁氧还蛋白-质醌还原酶(ferredoxin-plastoquinone reductase, FQR), 以依赖于PGR5的方式从Fd接收电子, 然后去还原PQ, 这条由PGRL1/PGR5介导的循环电子传递途径对抗霉素A敏感。另外一条途径则依赖于NAD(P)H脱氢酶(NDH)复合体(Burrows等1998; Shikanai等1998)。对叶绿体NDH复合体的研究最初是通过在质体中发现与线粒体复合体I的亚基高度同源的基因(*ndh*基因) (Ohyama等1988; Shinozaki等1986; Sugiura 1992)而开始的。线粒体NADH-泛醌氧化还原酶(复合体I)是位于呼吸链上游的一个多亚基蛋白复合体, 它将电子从NADH传递至泛醌, 并耦联跨膜质子梯度形成用于合成ATP (Friedrich等1995)。NDH复合体参与围绕PSI的循环电子传递的实验证据首先来自对蓝藻的研究(Mi等1995, 1994, 1992a, b)。通过对集胞蓝藻PCC6803的NDH-B失活突变体进行P700氧化还原和叶绿素荧光动力学研究, 首次证明了NDH不仅参与呼吸电子传递而且参与围绕PSI的循环电子传递。随后, 一系列对高等植物叶绿体*ndh*基因缺失突变体的分析工作也证明了NDH复合体参与围绕PSI的对抗霉素A不敏感的循环电子传递(Burrows等1998; Horváth等2000; Kofer等1998; Shikanai等1998)。此外, NDH位于高等植物间质类囊体膜上, 而这里被认为是PSI分布和进行循环电子传递的区域(Berger等1993; Burrows等1998; Endo等1997; Horváth等2000; Nixon等1989; Shikanai等1998)。由PGRL1/PGR5介导的循环电子传递途径主要功能是光保护和调节NADPH/ATP的比例, 而由NDH介导的循环电子传递途径主要是在阻止基质电子受体的过还原起着重要作用(Shikanai 2007)。两条途径在一定程度上互为补充。更为直接的证据来自于对拟南芥双突变体*crr2pgr5*、*crr3pgr5*和*crr4pgr5* (Munekage等2004)的研究。*crr2*、*crr3*和*crr4*突变体为拟南芥NDH的突变体, 在正常光强和高光强下都没有明显表型。*pgr5*突变体在正常光下虽然叶绿素含量比野生型较少, 但生长并不受影响, *crr2pgr5*、*crr3pgr5*和*crr4pgr5*双突变体在正常光下就表现为生长受到严重抑制, 强有力地说明了在叶绿体中, NDH复合体和PGR5分别介导两条围绕光系统I的循环电子传递途径, 这两条循环电子传递途径互为补充, 不能同时缺失。本综述主要就叶绿体NAD(P)H脱氢酶复合体的功能、结构、亚基组成及调控展开讨论。

1 叶绿体NDH复合体的生理功能

研究表明, NDH复合体在类囊体膜中只占PSII含量的1.5% (Burrows等1998)。因而, NDH复合体在高等植物中的作用曾长期被忽视。然而, 近十几年来的研究表明, NDH复合体在植物受到胁迫条件时具有重要的生理意义。

(1)参与循环电子传递。作用光关闭后叶绿素荧光瞬时上升是由光下积累的还原产物的电子在暗中还原PQ所致, 可以反映围绕光系统I的循环电

子传递(Asada等1993; Groom等1993)。烟草和拟南芥NDH复合体缺失突变体(Burrows等1998; Ishida等2009; Rumeau 2005; Shikanai等1998)在正常生长条件下与野生型没有显著可见的区别, 但是作用光关闭后叶绿素荧光瞬时上升这一现象都被明显抑制。这些结果说明了叶绿体中NDH复合体参与了基质电子库到PQ的电子传递。

(2)缓解基质过还原和氧化胁迫。这主要来自对质体*ndh*基因敲除的烟草的研究。Burrows等(1998)发现, 在轻微干旱条件下, NDH缺失突变体的非光化学猝灭能力降低。在强光下, 烟草*ndhB*突变体更容易被光漂白(Endo等1999)。Horváth等(2000)也观察到*ndhB*突变体在低湿下生长缓慢。Munné-Bosch等(2005)对烟草进行持续的干旱处理后发现, *ndhB*突变体的抗霉素A敏感的PSI循环电子传递途径上调。虽然拟南芥中低温诱导PGR5/PGRL1介导的循环电子途径, 而且NdhH在适应的低温条件下有所减低(Ivanov等2012), 但是在研究烟草*ndhC-J-K*突变体中发现, 在低温或高温胁迫下比野生型更容易积累活性氧(Wang等2006)。这些结果反应了NDH复合体参与减缓光氧化胁迫。Wu等(2011)发现低浓度$NaHSO_3$在暗光转化状态下促进NAD(P)H脱氢酶复合体介导的循环电子传递, 同时通过促进NAD(P)H脱氢酶复合体介导的光合磷酸化来减缓光氧化伤害, 从而提高光合作用(Wu等2012)。

(3)提供ATP来源。光化学反应中产生的ATP和NADPH, 在各种代谢途径中被利用, 然而主要是用于CO_2固定, 每同化一分子的CO_2所需要的ATP/NADPH的理论比例是1.5。然而, 在C_3植物中, 由于光呼吸的存在, 所需的ATP/NADPH的理论值增加到1.66, 非循环磷酸化在形成两分子NADPH的同时最多只能形成两分子ATP, 尚不能满足光合碳同化的需求。当植物体处于能量需求不同的各种发育阶段或面对环境变化时, 线性电子传递所产生的固定比例的ATP和NADPH往往不能满足上述条件之下CO_2同化的需求, 只产生ATP, 而不产生NADPH的循环电子传递途径可能可以提供多余的ATP (Shikanai 2007), 用于满足光合碳同化对ATP的需求。*hcef1*是一个果糖1,6-二磷酸酶突变体, 在这突变体中只有NDH介导的循环电子途径增强, 对*hcef*突变体的研究进一步说明了C_3植物光合作用中循环电子途径是满足ATP需求增加的关键步骤(Livingston等2010)。在C_4光合作用植物叶片中发现NDH表达量很高(Darie等2006; Kubicki等1996)。在NADP-苹果酸类型的C_4植物中, 维管束鞘细胞需要更多ATP, NDH复合体在其中积累更多。相反, 在NAD-苹果酸类型的C_4植物中, 叶肉细胞的ATP需求更多, NDH复合体在叶肉细胞中过量积累。这表明NDH复合体很可能在C_4光合作用中提供能量(Takabayashi 2005)。

2 叶绿体NDH复合体的亚基组成

大肠杆菌(*Escherichia coli*) NDH-1复合体是复合体I类酶的最小组成模式, 包括14个亚基(其中2个亚基融合成一个蛋白), 从NuoA到NuoN。这个最小的模式可以进行最基本的能量转化反应(Brandt 2006; Friedrich和Weiss 1997)。在叶绿体和蓝藻的基因组中比对, 只能发现11个与之同源的基因, 包括*ndhA~ndhK*。然而, 大肠杆菌中与NADH氧化相关的3个亚基NuoE、NuoF和NuoG在叶绿体和蓝藻中都没有找到同源蛋白。这3个亚基含有NADH结合位点、FMN结合位点和大量酶活性必需的铁硫簇。经过大量深入的研究, 仍没有发现可能的具有氧化NAD(P)H功能的亚基。这表明, 在叶绿体和蓝藻中可能已经进化出另外的催化结构域。到目前为止, 通过生物信息学、蛋白质组学和遗传学等多种手段, 在叶绿体中已经发现了28个NDH亚基。其中NdhA~K的11个亚基为叶绿体编码, 其余均为核编码。此外, NdhA~O这15个亚基在蓝藻中均有同源蛋白(Friedrich和Scheide 2000)。

综合各种突变体背景下复合体的稳定性和蛋白质组学结果, Ifuku等(2011)提出了一个叶绿体NDH结构模式, 这个复合体包括5个亚复合体。

(1)膜亚复合体, 包括NdhA~NdhG共7个亚基。这7个亚基在不同的NDH (如*E. coli*的NDH-1和线粒体的复合体I)中相对比较保守, 缺失这些亚基的突变体的研究表明膜亚复合体是维持整个复合体的稳定以及活性的关键所在, 例如缺失NdhB的突变体, 整个复合体几乎完全解聚(Hashimoto等2003)。

(2)亚复合体A, 包括NdhH~NdhO共8个亚基。嗜热链球菌NDH-1的亲水区域的晶体结构分

析中发现相对应NdhH、NdhK、NdhA的同源亚基Nqo4、Nqo6、Nqo8以及Nqo7、Nqo10、Nqo11(相对应膜亚复合体中NdhC、NdhG、NdhE亚基)的交界处的洞穴可能为质醌的结合位点(Efremov等2010), 而且在嗜热链球菌中NdhH~NdhK这4个亚基的同源蛋白结合3个铁硫簇, 参与电子传递过程(Sazanov和Hinchliffe 2006)。这表明叶绿体NDH和嗜热链球菌NDH-1中从NdhH~NdhK到质醌结合位点的电子传递通路是保守的。NdhL~NdhO对亚复合体A的稳定性也是必须的, 缺少这4个亚基, 亚复合体A几乎完全解聚, 表明它们对于NdhH~NdhK的组装和稳定性是重要的(Peng等2009; Rumeau 2005)。

(3)亚复合体B, 包括NDF1、NDF2、NDF4、NDF6和NDH18共5个亚基。这些亚基影响NDH的生成和复合体的稳定, 同时膜亚复合体也影响了这些亚基的积累(Ishikawa等2008; Peng等2009; Sirpiö等2009a; Suorsa等2010; Takabayashi等2009)。如果其中的亚基缺失, 积累的亚复合体A的水平只有野生型的10% (Peng等2009; Yabuta等2010), 所以把他们认为是NDH的组成成分。而且经预测, NDF4含有一个铁硫簇结合区域, NDF4的铁硫簇可能是氧化还原活性部位, 参与到NDH的电子传递(Takabayashi等2009)。

(4)类囊体腔亚复合体, 包括PPL2、CYP20-2、FKBP16-2、PQL1和PQL2共5个亚基(Ishihara等2007; Peng等2009; Sirpiö等2009b; Yabuta等2010)。这些亚基影响了NDH复合体亚基活性、组装以及稳定, 这个亚复合体不存在时, 亚复合体A几乎完全解聚, 而亚复合体B也只有正常水平的25%~50% (Peng等2009)。在分离的拟南芥的NDH-PSI超复合体中能检测到CYP20-2 (Sirpiö等2009b), 但是有些亚基仍需进一步证实是否与NDH有结构上的联系。所以这个亚复合体是否是NDH的组成仍需进一步证明。

(5)电子供体结合亚基, 包括CRR31(NdhS)、CRRJ(NdhT)和CRRL(NdhU)共3个亚基。CRR31与CRRJ和CRRL相互作用, CRRJ和CRRL含有跨膜区域。CRR31亚基的C端具有类SH3 (Src homology)结构域, 具有Fd结合位点。体外的Fd依赖的质醌还原系统证明, 这些亚基是结合Fd和催化活性所必需的(Yamamoto等2011)。蓝藻的NdhS的C端是保守的, 说明NDH的这个电子供体元件是保守的(Battchikova等2012)。

与NDH相关的蛋白中, 还有些蛋白被推测是NDH的组成亚基, 如CRR3。CRR3蛋白具有一个跨膜区域, 在*crr3*的突变体中, NdhH缺失, 而在NDH的其他突变体(*crr6*和*crr7*)中也检测不到CRR3蛋白的积累, 所以推测CRR3可能是叶绿体NDH亚复合体A或假定的“催化亚复合体”的一个亚基(Muraoka等2006)。但是在NDH-PSI超复合体的质谱分析中没有发现有CRR3蛋白(Peng等2009)。

3 NDH复合体的调控

除了上面提到的NDH复合体的组成亚基外, 目前还发现了很多不直接参与NDH结构组成的蛋白, 这些蛋白主要负责调控NDH复合体亚基的合成、组装和维持NDH复合体的稳定。

3.1 对叶绿体*Ndh* mRNA的调控

叶绿体中NDH复合体亚基数目较多, 其中NdhA~K的11个亚基为叶绿体编码。其余均为核编码。而叶绿体基因的表达调控主要是在转录后水平, 包括mRNA的剪切、编辑、加工和降解。由于叶绿体基因不编码调控蛋白, 所以叶绿体基因的表达调控基本是由核基因来完成(表1)。NdhA~K这11个亚基分别属于4个转录单位: *ndhF*、*ndhB*、*ndhH/A/I/G/E/D*和*ndhC/K/J* (Yukawa和Sugiura 2008)。目前对*ndh*基因转录和翻译的调控研究有了很大的进展(del Campo等2000; Favory等2005; Hirose和Sugiura 1997; Maria del Campo等2006; Okuda等2008; Schmitz-Linneweber等2001; Yukawa和Sugiura 2008)。目前大部分NDH膜组分亚基的转录调控是由一类PPR (pentatricopeptide repeat)蛋白参与的。PPR蛋白是一类由35个氨基酸的序列单元经串联重复组成的蛋白, 主要参与叶绿体和线粒体基因的转录后调控。其中CRR2参与*ndhB*和*rps7*基因之间的剪切, 这对*ndhB*基因的翻译是必需的(Hashimoto等2003)。一个sigma因子Sig4被认为参与*ndhF*基因的转录(Favory等2005)。PPR蛋白CRR4在参与*ndhD*基因的起始密码子的编辑中, 为*ndhD*的转录因子结合识别起着重要作用(Kotera等2005; Okuda等2007)。除CRR4外, PPR蛋白CRR21负责*ndhD*基因的第2个位点的编辑, 将胞嘧啶转化

表1 拟南芥中参与调控叶绿体Ndh mRNA的核基因编码蛋白

Table 1 *Arabidopsis* nuclear-encoded proteins involved in regulating of chloroplast Ndh mRNA

亚基	基因编号	调控蛋白	基因编号	功能	参考文献
NdhA	ATcG01100	PGR3	AT4G31850	影响*ndhA* mRNA的稳定	Cai等2011
NdhB	ATcG00890, ATcG01250	CRR2	AT3G46790	参与*ndhB* mRNA的剪切	Hashimoto等2003; Okuda等2009
		CRR22	AT1G11290	参与*ndhB* mRNA的编辑	Okuda等2009
		CRR28	AT1G59720	参与*ndhB* mRNA的编辑	Okuda等2009
NdhD	ATcG01050	CRR4	AT2G45350	参与*ndhD* mRNA的编辑	Kotera等2005; Okuda等2007
		CRR21	AT5G55740	参与*ndhD* mRNA的编辑	Okuda等2007
		CRR22	AT1G11290	参与*ndhD* mRNA的编辑	Okuda等2009
		CRR28	AT1G59720	参与*ndhD* mRNA的编辑	Okuda等2009
NdhF	ATcG01010	Sig4	AT5G13730	影响*ndhF*基因的转录	Favory等2005
		CP31A	AT4G24770	参与*ndhF* mRNA的编辑	Tillich等2009; Kupsch等2012

成尿嘧啶, 从而将NdhD亚基的128位的丝氨酸转化为亮氨酸(Okuda等2007)。同样, PPR蛋白CRR22参与*ndhB*-7和*ndhD*-5的RNA编辑, 使得NdhB的249位的丝氨酸转变成苯丙氨酸, NdhD的296位脯氨酸转变成亮氨酸。CRR28则编辑*ndhB*-2和*ndhD*-3位点, 分别将156位的脯氨酸转化为亮氨酸和将293位的丝氨酸转化为亮氨酸(Okuda等2009)。预测这几个氨基酸都处于跨膜螺旋中, *crr21*、*crr22*和*crr28*突变体NDH活性完全丢失, 表明NdhB、NdhD和NdhF对于维持NDH的活性和稳定性是关键的。烟草CP31之前就被发现参与*ndhB* mRNA的编辑(Hirose和Sugiura 2001), 近来发现, 拟南芥同源基因CP31A对*ndhF* mRNA的编辑是必需的, 而且突变体*cp31a*中*ndhB*和*ndhD* mRNA的编辑效率也有所减弱。同时, 拟南芥中另一个核蛋白CP31B对编辑也起作用(Tillich等2009)。最新的研究还发现CP31A对于防止*ndhF* mRNA被3′外切酶降解是必需的(Kupsch等2012)。PGR3是一个核编码的PPR蛋白, 3个*pgr3*突变体中, 有2个突变体中的NDH的活性和Ndh亚基的积累都受损(Yamazaki等2004), 进一步研究发现PGR3与*ndhA*的mRNA的5′UTR特异结合, 对于RNA的稳定起着重要作用(Cai等2011)。

3.2 对NDH复合体的组装以及稳定的调控

除了参与叶绿体*ndh* mRNA的编辑剪切的核编码的蛋白外, 还发现一些影响NDH复合体的组装和功能以及稳定的蛋白(表2)。例如, 目前研究已发现了蛋白CRR1、CRR6、CRR7、CRR41和CRR42等参与了亚复合体A的组装(Peng等2012)。拟南芥的CRR1蛋白定位在基质中(Shimizu和Shikanai 2007), 由于CRR1蛋白在*crr2*突变体中没变化, 所以被认为是NDH的辅助蛋白而非组成蛋白, 辅助NDH复合体的发生和稳定(Shimizu和Shikanai 2007)。Munshi等(2005, 2006)通过NDH活性分析,

表2 拟南芥中参与NDH复合体的组装以及稳定的调控蛋白

Table 2 Proteins involved in assembly and stabilization of NDH complex in *Arabidopsis*

亚复合体	调控蛋白	基因编号	功能	文献
亚复合体A	CRR1	AT5G52100	参与亚复合体A的组装, 辅助NDH复合体的发生和稳定	Shimizu和Shikanai 2007
	CRR6	AT2G47910	参与亚复合体A的组装	Munshi等2006; Peng等2010
	CRR7	AT5G39210	参与亚复合体A的组装	Munshi等2005; Peng等2010
	CRR41	At1g51100	参与亚复合体A的组装	Peng等2012
	CRR42	At5g20935	参与亚复合体A的组装	Peng等2012
	Cpn60β4	At1g26230	参与NdhH亚基的折叠	Peng等2011
	HCF101	AT3G24430	参与亚复合体A的组装	Peng等2012
	NDF5	AT1G55370	影响亚复合体A的稳定	Ishida等2009
亚复合体B	NDF5	AT1G55370	影响亚复合体B的稳定	Ishida等2009

鉴定到2个失去NDH活性的*crr6*和*crr7*突变体。这2个蛋白是NDH复合体的亚复合体A组装所必需的。在突变体中, 编码亚复合体A的质体基因的转录并没有受影响, 因此CRR6和CRR7可能参与亚复合体A组装(Peng等2010, 2012)。通过突变体*crr27/cpn60β4*和*cpn60α*的研究发现Cpn60复合体参与NdhH亚基的折叠(Peng等2011), 继而参与亚复合体的A的组装(Peng等2012)。近来又在拟南芥中发现了2个叶绿体基质蛋白CRR41和CRR42参与到了亚复合体的A的组装, 并通过免疫共沉淀等方法发现HCF101也有可能参与到了亚复合体A的组装(Peng等2012)。NDH复合体基因的共表达分析鉴定到一个蛋白NDF5, 在NDH-PSI超复合体的质谱分析没有检测到(Peng等2009), 表明NDF5并不是NDH复合体的一个亚基。但是在*ndf5*突变体中, 亚复合体A和亚复合体B的稳定性严重影响。由于NDF5在*ndho*突变体中过量积累, 表明NDF5可能结合到亚复合体B, 然后招募亚复合体A到合适位置(Ishida等2009)。高等植物特有的*PIFI*基因与*NDH*的基因共表达(Wang和Portis 2007), 在*pifi*突变体中NdhH亚基是正常表达的, 而且在*crr2*突变体中PIFI蛋白水平也接近野生型。而且, *pifi*突变体在高光条件下非光化学猝灭系数较低, 推测PIFI是NDH的一个辅助蛋白, 可能参与到NDH介导的叶绿体呼吸途径(Wang和Portis 2007)。

4 NDH与PSI形成超复合体

早期的关于叶绿体NDH复合体的报道认为, NDH复合体分子量约为550 kDa (Burrows等1998; Quiles等2002)。2005年, 两个实验室分别在拟南芥和玉米中检测到高分子量(超过1 000 kDa)的NDH复合体(Aro 2004; Darie等2005)。当时认为这个大的复合体可能仅仅是NDH复合体形成的二聚体。Peng等(2009)认为, 在高等植物中, NDH复合体与PSI结合后才形成了这个新的超复合体。拟南芥*lhca5*或*lhca6*突变体中的这个超复合体就不存在, 取而代之的是一个小一点的复合体, 有可能这个小一点的复合体只含有一个拷贝的NDH和PSI, 进一步实验发现在*lhca5lhca6*双突变体中NDH复合体是以单体形式存在, 说明完整的NDH复合体(分子量约700 kDa)通过Lhca5和Lhca6与2个拷贝的PSI (分子量约530 kDa)结合, 形成一个超大复合体(Peng和Shikanai 2011)。*lhca5lhca6*双突变体的叶绿素荧光分析发现超复合体的形成对NDH的活性并不是必须的, 双突变体中NDH的亚基的水平与野生型相比较有所下降, 尤其是高光强随着时间的增加逐渐下降。所以超复合体对于NDH复合体的稳定是必需的, 尤其是对于在高光胁迫下(Peng和Shikanai 2011)。虽然在蓝藻和地钱中也都发现了超复合体的存在(Kubota等2010; Ueda等2012), 但是都没发现有Lhca5和Lhca6的同源蛋白的存在(Ueda等2012)。

5 展望

关于叶绿体复合体的结构和亚基组成及调控在最近几年内取得重要进展, 大肠杆菌和嗜热杆菌呼吸链复合体I的晶体结构的解析也对研究类囊体膜NDH复合体的研究有很大影响, 但是仍然有很多问题需要解决。如果能从高等植物中分离纯化NDH复合体, 继而得到晶体结构对NDH复合体的研究是很有意义的, 这样更能深入而且全面地理解NDH复合体各个亚基的功能和反应机理。近年高等植物中对NDH的研究很大一部分是在寻找新亚基或调控蛋白上, 虽然也已经找到很多新亚基和参与调控的蛋白, 但是负责结合和氧化NADH和NADPH的亚基还未清楚, 而且仍然有很多亚基的调控蛋白是空缺的, 对组装及其调控也需进一步研究。NDH在不同生理条件下的光合响应机制方面研究并不够深入。蓝藻中有不同形式的具有不同功能NDH-1复合体, 但是其反应机制是否相同并不清楚, 叶绿体NDH复合体是否也存在不同形式的NDH复合体？起着不同的功能？是否是对不同环境起着不同作用？这些问题都有待解决。

参考文献

米华玲(2003). 类囊体膜NAD(P)H脱氢酶复合体及其在电子传递中的功能. 见: 匡廷云主编. 光合作用原初光能转化过程的原理与调控. 南京: 江苏科学技术出版社, 371~392

Aro EM (2004). Dynamics of photosystem II: a proteomic approach to thylakoid protein complexes. J Exp Bot, 56: 347~356

Asada K, Heber U, Schreiber U (1993). Electron flow to the intersystem chain from stromal components and cyclic electron flow in maize chloroplasts, as detected in intact leaves by monitoring redox change of P700 and chlorophyll fluorescence. Plant Cell Physiol, 34: 39~50

Battchikova N, Wei L, Du L, Bersanini L, Aro E-M, Ma W (2012). Identification of a novel Ssl0352 Protein (NdhS), essential for ef-

ficient operation of cyclic electron transport around photosystem I, in NADPH:plastoquinone oxidoreductase (NDH-1) complexes of *Synechocystis* sp. PCC 6803. J Biol Chem, 287: 8660

Bendall DS, Manasse RS (1995). Cyclic photophosphorylation and electron transport. Biochim Biophys Acta, 1229: 23~38

Berger S, Ellersiek U, Westhoff P, Steinmuller K (1993). Studies on the expression of Ndh-H, a subunit of the NAD(P)H-plastoquinone-oxidoreductase of higher-plant chloroplasts. Planta, 190: 25~31

Brandt U (2006). Energy converting NADH: quinone oxidoreductase (complex I). Annu Rev Biochem, 75: 69~92

Burrows PA, Sazanov LA, Svab Z, Maliga P, Nixon PJ (1998). Identification of a functional respiratory complex in chloroplasts through analysis of tobacco mutants containing disrupted plastid *ndh* genes. EMBO J, 17: 868~876

Cai W, Okuda K, Peng L, Shikanai T (2011). PROTON GRADIENT REGULATION 3 recognizes multiple targets with limited similarity and mediates translation and RNA stabilization in plastids. Plant J, 67: 318~327

Dalcorso G, Pesaresi P, Masiero S, Aseeva E, Schunemann D, Finazzi G, Joliot P, Barbato R, Leister D (2008). A complex containing PGRL1 and PGR5 is involved in the switch between linear and cyclic electron flow in *Arabidopsis*. Cell, 132: 273~285

Darie CC, Biniossek ML, Winter V, Mutschler B, Haehnel W (2005). Isolation and structural characterization of the Ndh complex from mesophyll and bundle sheath chloroplasts of *Zea mays*. FEBS J, 272: 2705~2716

Darie CC, De Pascalis L, Mutschler B, Haehnel W (2006). Studies of the Ndh complex and photosystem II from mesophyll and bundle sheath chloroplasts of the C_4-type plant *Zea mays*. J Plant Physiol, 163: 800~808

del Campo EM, Sabater B, Martin M (2000). Transcripts of the *ndhH-D* operon of barley plastids: possible role of unedited site III in splicing of the ndhA intron. Nucleic Acids Res, 28: 1092~1098

Efremov RG, Baradaran R, Sazanov LA (2010). The architecture of respiratory complex I. Nature, 465: 441~445

Endo T, Mi HL, Shikanai T, Asada K (1997). Donation of electrons to plastoquinone by NAD(P)H dehydrogenase and by ferredoxin-quinone reductase in spinach chloroplasts. Plant Cell Physiol, 38: 1272~1277

Endo T, Shikanai T, Takabayashi A, Asada K, Sato F (1999). The role of chloroplastic NAD(P)H dehydrogenase in photoprotection. FEBS Lett, 457: 5~8

Favory JJ, Kobayshi M, Tanaka K, Peltier G, Kreis M, Valay JG, Lerbs-Mache S (2005). Specific function of a plastid sigma factor for *ndhF* gene transcription. Nucleic Acids Res, 33: 5991~5999

Feild TS, Nedbal L, Ort DR (1998). Nonphotochemical reduction of the plastoquinone pool in sunflower leaves originates from chlororespiration. Plant Physiol, 116: 1209~1218

Friedrich T, Scheide D (2000). The respiratory complex I of bacteria, archaea and eukarya and its module common with membrane-bound multisubunit hydrogenases. FEBS Lett, 479: 1~5

Friedrich T, Steinmuller K, Weiss H (1995). The proton-pumping respiratory complex I of bacteria and mitochondria and its homologue in chloroplasts. FEBS Lett, 367: 107~111

Friedrich T, Weiss H (1997). Modular evolution of the respiratory NADH:ubiquinone oxidoreductase and the origin of its modules. J Theor Biol, 187: 529~540

Groom QJ, Kramer DM, Crofts AR, Ort DR (1993). The nonphotochemical reduction of plastoquinone in leaves. Photosynth Res, 36: 205~215

Hashimoto M, Endo T, Peltier G, Tasaka M, Shikanai T (2003). A nucleus-encoded factor, CRR2, is essential for the expression of chloroplast *ndhB* in *Arabidopsis*. Plant J, 36: 541~549

Heber U, Walker D (1992). Concerning a dual function of coupled cyclic electron transport in leaves. Plant Physiol, 100: 1621~1626

Hertle AP, Blunder T, Wunder T, Pesaresi P, Pribil M, Armbruster U, Leister D (2013). PGRL1 is the elusive ferredoxin-plastoquinone reductase in photosynthetic cyclic electron flow. Mol Cell, 49: 511~523

Hirose T, Sugiura M (1997). Both RNA editing and RNA cleavage are required for translation of tobacco chloroplast *ndhD* mRNA: a possible regulatory mechanism for the expression of a chloroplast operon consisting of functionally unrelated genes. EMBO J, 16: 6804~6811

Hirose T, Sugiura M (2001). Involvement of a site-specific trans-acting factor and a common RNA-binding protein in the editing of chloroplast mRNAs: development of a chloroplast *in vitro* RNA editing system. EMOB J, 20: 1144~1152

Horváth EM, Peter SO, Joet T, Rumeau D, Cournac L, Horvath GV, Kavanagh TA, Schafer C, Peltier G, Medgyesy P (2000). Targeted inactivation of the plastid *ndhB* gene in tobacco results in an enhanced sensitivity of photosynthesis to moderate stomatal closure. Plant Physiol, 123: 1337~1350

Ifuku K, Endo T, Shikanai T, Aro EM (2011). Structure of the chloroplast NADH dehydrogenase-like complex: Nomenclature for nuclear-encoded subunits. Plant Cell Physiol, 52: 1560~1568

Ishida S, Takabayashi A, Ishikawa N, Hano Y, Endo T, Sato F (2009). A novel nuclear-encoded protein, NDH-dependent cyclic electron flow 5, is essential for the accumulation of chloroplast NAD(P)H dehydrogenase complexes. Plant Cell Physiol, 50: 383~393

Ishihara S, Takabayashi A, Ido K, Endo T, Ifuku K, Sato F (2007). Distinct functions for the two PsbP-like proteins PPL1 and PPL2 in the chloroplast thylakoid lumen of *Arabidopsis*. Plant Physiol, 145: 668~679

Ishikawa N, Takabayashi A, Ishida S, Hano Y, Endo T, Sato F (2008). NDF6: a thylakoid protein specific to terrestrial plants is essential for activity of chloroplastic NAD(P)H dehydrogenase in *Arabidopsis*. Plant Cell Physiol, 49: 1066~1073

Ivanov AG, Rosso D, Savitch LV, Stachula P, Rosembert M, Oquist G, Hurry V, Huner NPA (2012). Implications of alternative electron sinks in increased resistance of PSII and PSI photochemistry to high light stress in cold-acclimated *Arabidopsis thaliana*. Photosynth Res, 113: 191~206

Joet T, Genty B, Josse EM, Kuntz M, Cournac L, Peltier G (2002). Involvement of a plastid terminal oxidase in plastoquinone oxi-

dation as evidenced by expression of the *Arabidopsis thaliana* enzyme in tobacco. J Biol Chem, 277: 31623~31630

Kofer W, Koop HU, Wanner G, Steinmuller K (1998). Mutagenesis of the genes encoding subunits A, C, H, I, J and K of the plastid NAD(P)H-plastoquinone-oxidoreductase in tobacco by polyethylene glycol-mediated plastome transformation. Mol Gen Genet, 258: 166~173

Kotera E, Tasaka M, Shikanai T (2005). A pentatricopeptide repeat protein is essential for RNA editing in chloroplasts. Nature, 433: 323~326

Kubicki A, Funk E, Westhoff P, Steinmüller K (1996). Differential expression of plastome-encoded *ndh* genes in mesophyll and bundle-sheath chloroplasts of the C_4 plant *Sorghum bicolor* indicates that the complex I-homologous NAD(P)H-plastoquinone oxidoreductase is involved in cyclic electron transport. Planta, 199: 276~281

Kubota H, Sakurai I, Katayama K, Mizusawa N, Ohashi S, Kobayashi M, Zhang PP, Aro EM, Wada H (2010). Purification and characterization of photosystem I complex from *Synechocystis* sp. PCC 6803 by expressing histidine-tagged subunits. Biochim Biophys Acta, 1797: 98~105

Kupsch C, Ruwe H, Gusewski S, Tillich M, Small I, Schmitz-Linneweber C (2012). *Arabidopsis* chloroplast RNA binding proteins CP31A and CP29A associate with large transcript pools and confer cold stress tolerance by influencing multiple chloroplast RNA processing steps. Plant Cell, 24: 4266~4280

Livingston AK, Cruz JA, Kohzuma K, Dhingra A, Kramer DM (2010). An *Arabidopsis* mutant with high cyclic electron flow around photosystem I (hcef) involving the NADPH dehydrogenase complex. Plant Cell, 22: 221~233

Maria del Campo E, Sabater B, Martin M (2006). Characterization of the 5'- and 3'-ends of mRNAs of *ndhH*, *ndhA* and *ndhI* genes of the plastid *ndhH-D* operon. Biochimie, 88: 347~357

Martin M, Casano LM, Zapata JM, Guera A, del Campo EM, Schmitz-Linneweber C, Maier RM, Sabater B (2004). Role of thylakoid Ndh complex and peroxidase in the protection against photo-oxidative stress: fluorescence and enzyme activities in wild-type and *ndhF*-deficient tobacco. Physiol Plant, 122: 443~452

Mi H, Endo T, Ogawa T, Asada K (1995). Thylakoid membrane-bound, NADPH-specific pyridine nucleotide dehydrogenase complex mediates cyclic electron transport in the cyanobacterium *Synechocystis* sp. PCC 6803. Plant Cell Physiol, 36: 661~668

Mi H, Endo T, Schreiber U, Asada K (1992a). Donation of electrons from cytosolic components to the intersystem chain in the cyanobacterium *synechococcus* sp. PCC 7002 as determined by the reduction of $P700^+$. Plant Cell Physiol, 33: 1099~1105

Mi H, Endo T, Schreiber U, Ogawa T, Asada K (1992b). Electron donation from cyclic and respiratory flows to the photosynthetic intersystem chain is mediated by pyridine nucleotide dehydrogenase in the cyanobacterium *Synechocystis* PCC 6803. Plant Cell Physiol, 33: 1233~1237

Mi H, Endo T, Schreiber U, Ogawa T, Asada K (1994). NAD(P)H dehydrogenase-dependent cyclic electron flow around photosystem-I in the cyanobacterium *Synechocystis* PCC 6803: a study of dark-starved cells and spheroplasts. Plant Cell Physiol, 35: 163~173

Munekage Y, Hashimoto M, Miyake C, Tomizawa K-I, Endo T, Tasaka M, Shikanai T (2004). Cyclic electron flow around photosystem I is essential for photosynthesis. Nature, 429: 579~582

Munekage Y, Hojo M, Meurer J, Endo T, Tasaka M, Shikanai T (2002). *PGR5* is involved in cyclic electron flow around photosystem I and is essential for photoprotection in *Arabidopsis*. Cell, 110: 361~371

Munné-Bosch S, Shikanai T, Asada K (2005). Enhanced ferredoxin-dependent cyclic electron flow around photosystem I and α-tocopherol quinone accumulation in water-stressed ndhB-inactivated tobacco mutants. Planta, 222: 502~511

Munshi K, Kobayashi Y, Shikanai T (2005). Identification of a novel protein, CRR7, required for the stabilization of the chloroplast NAD(P)H dehydrogenase complex in *Arabidopsis*. Plant J, 44: 1036~1044

Munshi MK, Kobayashi Y, Shikanai T (2006). Chlororespiratory reduction 6 is a novel factor required for accumulation of the chloroplast NAD(P)H dehydrogenase complex in *Arabidopsis*. Plant Physiol, 141: 737~744

Muraoka R, Okuda K, Kobayashi Y, Shikanai T (2006). A eukaryotic factor required for accumulation of the chloroplast NAD(P)H dehydrogenase complex in *Arabidopsis*. Plant Physiol, 142: 1683~1689

Nixon PJ, Gounaris K, Coomber SA, Hunter CN, Dyer TA, Barber J (1989). *psbG* is not a photosystem two gene but may be an *ndh* gene. J Biol Chem, 264: 14129~14135

Ohyama K, Kohchi T, Sano T, Yamada Y (1988). Newly identified groups of genes in chloroplasts. Trends Biochem Sci, 13: 19~22

Okuda K, Chateigner-Boutin AL, Nakamura T, Delannoy E, Sugita M, Myouga F, Motohashi R, Shinozaki K, Small I, Shikanai T (2009). Pentatricopeptide repeat proteins with the DYW motif have distinct molecular functions in RNA editing and RNA cleavage in *Arabidopsis* chloroplasts. Plant Cell, 21: 146~156

Okuda K, Habata Y, Kobayashi Y, Shikanai T (2008). Amino acid sequence variations in *Nicotiana* CRR4 orthologs determine the species-specific efficiency of RNA editing in plastids. Nucleic Acids Res, 36: 6155~6164

Okuda K, Myouga F, Motohashi R, Shinozaki K, Shikanai T (2007). Conserved domain structure of pentatricopeptide repeat proteins involved in chloroplast RNA editing. Proc Natl Acad Sci USA, 104: 8178~8183

Peng L, Cai W, Shikanai T (2010). Chloroplast stromal proteins, CRR6 and CRR7, are required for assembly of the NAD(P)H dehydrogenase subcomplex A in *Arabidopsis*. Plant J, 63: 203~211

Peng L, Fukao Y, Fujiwara M, Shikanai T (2012). Multistep assembly of chloroplast NADH dehydrogenase-like subcomplex A requires several nucleus-encoded proteins, including CRR41 and CRR42, in *Arabidopsis*. Plant Cell, 24: 202~214

Peng L, Fukao Y, Fujiwara M, Takami T, Shikanai T (2009). Efficient operation of NAD(P)H dehydrogenase requires supercomplex formation with photosystem I via minor LHCI in *Arabidopsis*. Plant Cell, 21: 3623~3640

Peng L, Shikanai T (2011). Supercomplex formation with photosystem I is required for the stabilization of the chloroplast NADH dehydrogenase-like complex in *Arabidopsis*. Plant Physiol, 155: 1629~1639

Peng LW, Fukao Y, Myouga F, Motohashi R, Shinozaki K, Shikanai T (2011). A chaperonin subunit with unique structures is essential for folding of a specific substrate. PLoS Biol, 9

Quiles MJ, Molina NC, Cuello J (2002). Isolation of an NADH dehydrogenase complex not associated to ferredoxin-$NADP^+$ oxidoreductase from oat stroma thylakoids. J Plant Physiol, 159: 457~464

Rumeau D (2005). New subunits NDH-M, -N, and -O, encoded by nuclear genes, are essential for plastid Ndh complex functioning in higher plants. Plant Cell, 17: 219~232

Sazanov LA, Hinchliffe P (2006). Structure of the hydrophilic domain of respiratory complex I from *Thermus thermophilus*. Science, 311: 1430~1436

Schmitz-Linneweber C, Tillich M, Herrmann RG, Maier RM (2001). Heterologous, splicing-dependent RNA editing in chloroplasts: allotetraploidy provides trans-factors. EMBO J, 20: 4874~4883

Shikanai T (2007). Cyclic electron transport around photosystem I: Genetic approaches. Annu Rev Plant Biol, 58: 199~217

Shikanai T, Endo T, Hashimoto T, Yamada Y, Asada K, Yokota A (1998). Directed disruption of the tobacco *ndhB* gene impairs cyclic electron flow around photosystem I. Proc Natl Acad Sci USA, 95: 9705~9709

Shimizu H, Shikanai T (2007). Dihydrodipicolinate reductase-like protein, CRR1, is essential for chloroplast NAD(P)H dehydrogenase in *Arabidopsis*. Plant J, 52: 539~547

Shinozaki K, Ohme M, Tanaka M, Wakasugi T, Hayashida N, Matsubayashi T, Zaita N, Chunwongse J, Obokata J, Yamaguchi-Shinozaki K et al (1986). The complete nucleotide sequence of the tobacco chloroplast genome: its gene organization and expression. EMBO J, 5: 2043~2049

Sirpiö S, Allahverdiyeva Y, Holmstrom M, Khrouchtchova A, Haldrup A, Battchikova N, Aro EM (2009a). Novel nuclear-encoded subunits of the chloroplast NAD(P)H dehydrogenase complex. Biol Chem, 284: 905~912

Sirpiö S, Holmström M, Battchikova N, Aro E-M (2009b). AtCYP20-2 is an auxiliary protein of the chloroplast NAD(P)H dehydrogenase complex. FEBS Lett, 583: 2355~2358

Sugiura M (1992). The chloroplast genome. Plant Mol Biol, 19: 149~168

Suorsa M, Sirpio S, Paakkarinen V, Kumari N, Holmstrom M, Aro EM (2010). Two proteins homologous to PsbQ are novel subunits of the chloroplast NAD(P)H dehydrogenase. Plant Cell Physiol, 51: 877~883

Takabayashi A (2005). Differential use of two cyclic electron flows around photosystem I for driving CO_2-concentration mechanism in C_4 photosynthesis. Proc Natl Acad Sci USA, 102: 16898~16903

Takabayashi A, Ishikawa N, Obayashi T, Ishida S, Obokata J, Endo T, Sato F (2009). Three novel subunits of *Arabidopsis* chloroplastic NAD(P)H dehydrogenase identified by bioinformatic and reverse genetic approaches. Plant J, 57: 207~219

Tillich M, Hardel SL, Kupsch C, Armbruster U, Delannoy E, Gualberto JM, Lehwark P, Leister D, Small ID, Schmitz-Linneweber C (2009). Chloroplast ribonucleoprotein CP31A is required for editing and stability of specific chloroplast mRNAs. Proc Natl Acad Sci USA, 106: 6002~6007

Ueda M, Kuniyoshi T, Yamamoto H, Sugimoto K, Ishizaki K, Kohchi T, Nishimura Y, Shikanai T (2012). Composition and physiological function of the chloroplast NADH dehydrogenase-like complex in *Marchantia polymorpha*. Plant J, 72: 683~693

Wang D, Portis AR Jr (2007). A novel nucleus-encoded chloroplast protein, PIFI, is involved in NAD(P)H dehydrogenase complex-mediated chlororespiratory electron transport in *Arabidopsis*. Plant Physiol, 144: 1742~1752

Wang P, Duan W, Takabayashi A, Endo T, Shikanai T, Ye JY, Mi H (2006). Chloroplastic NAD(P)H dehydrogenase in tobacco leaves functions in alleviation of oxidative damage caused by temperature stress. Plant Physiol, 141: 465~474

Wu YX, He W, Ma WM, Shen YK, Mi HL (2012). Low concentrations of $NaHSO_3$ enhance NAD(P)H dehydrogenase-dependent cyclic photophosphorylation and alleviate the oxidative damage to improve photosynthesis in tobacco. Chin Sci Bull, 57: 3872~3877

Wu YX, Zheng FF, Ma WM, Han ZG, Gu Q, Shen YK, Mi HL (2011). Regulation of NAD(P)H dehydrogenase-dependent cyclic electron transport around PSI by $NaHSO_3$ at low concentrations in tobacco chloroplasts. Plant Cell Physiol, 52: 1734~1743

Yabuta S, Ifuku K, Takabayashi A, Ishihara S, Ido K, Ishikawa N, Endo T, Sato F (2010). Three PsbQ-Like proteins are required for the function of the chloroplast NAD(P)H dehydrogenase complex in *Arabidopsis*. Plant Cell Physiol, 51: 866~876

Yamamoto H, Peng L, Fukao Y, Shikanai T (2011). An Src homology 3 domain-like fold protein forms a ferredoxin binding site for the chloroplast NADH dehydrogenase-like complex in *Arabidopsis*. Plant Cell, 23: 1480~1493

Yamazaki H, Tasaka M, Shikanai T (2004). PPR motifs of the nucleus-encoded factor, PGR3, function in the selective and distinct steps of chloroplast gene expression in *Arabidopsis*. Plant J, 38: 152~163

Yukawa M, Sugiura M (2008). Termination codon-dependent translation of partially overlapping *ndhC-ndhK* transcripts in chloroplasts. Proc Natl Acad Sci USA, 105: 19550~19554