特约综述 Invited Review

类囊体膜NAD(P)H脱氢酶复合体调控光合作用的研究进展

米华玲*

中国科学院上海生命科学研究院植物生理生态研究所, 植物分子遗传国家重点实验室, 光合作用与环境生物学实验室, 上海200032

摘要: 光合作用的光反应是由类囊体膜上有序排列的蛋白复合体高效驱动的, 除了光系统I、光系统II、细胞色素*b6/f*复合体、ATP合酶这四大复合体之外, NDH(P)H脱氢酶(NDH)复合体介导循环电子传递, 能保护植物免受各种胁迫环境条件下引起的光抑制, 在维持高效的光合作用中发挥重要的作用, 成为类囊体膜第五大蛋白复合体。目前, 对于NDH的组成、组装与生理功能等有了比较多的研究, 而对于NDH在光合作用的调控机理研究还有待于深入。因此, 了解NDH的作用机制对于揭示高效光合作用的运转机理, 具有重要的意义。本文着重对模式植物蓝藻和拟南芥NDH近期研究进展进行介绍, 总结NDH在光合机构运转中的调节作用, 并对今后的研究进行了展望。

关键词: NAD(P)H脱氢酶复合体; 循环电子传递; 光合作用; 叶绿体; 蓝藻

绿色植物包括蓝藻、绿藻和高等植物的光合作用光反应是在两个光化学系统(光系统I和光系统II)的参与下进行的。光系统II反应中心叶绿素680被光激发产生电荷分离后, 从分子水中夺取电子, 导致水分子裂解而放出氧气和质子。来自分子水的电子通过类囊体膜中一系列电子递体向光系统I传递, 最终使$NADP^+$还原为NADPH, 与此同时, 在膜中定向地进行电子传递, 使得类囊体膜内外产生质子浓度差和膜电位差, 驱动ATP合酶合成ATP, 此过程的电子传递为非循环电子传递(即线性电子传递), 所耦联的磷酸化过程为非循环磷酸化; 另外, 光系统I还原侧的电子还可以向两个光系统之间的电子递体进行传递, 形成闭环状电子传递, 这种电子传递途径被称为围绕光系统I的循环电子传递。围绕光系统I的循环电子传递也耦联ATP的形成, 但是, 没有NADPH或者其他还原物质的积累。循环电子传递产生ATP的过程被称为循环光合磷酸化。光反应产生的NADPH和ATP用于暗反应(光合碳同化)的CO_2固定。与暗反应相比, 光反应的光能利用率非常高, 光反应由在类囊体膜上有序排列的蛋白复合体驱动, 参与线性电子传递及非循环磷酸化的复合体包括光系统I、光系统II、细胞色素*b6/f*复合体、ATP合酶这四大复合体, 而参与循环电子传递和循环磷酸化的复合体还包括NDH(P)H脱氢酶[NAD(P)H dehydrogenase, NDH]复合体。研究表明, NDH介导的循环电子传递(Mi等1992, 1994, 1995; Shikanai等1998), 能保护植物免受各种胁迫环境条件下引起的光抑制(Endo等1999; Mi等2001; Wang等2006), 在维持高效的光合作用发挥了重要的作用(Munekage等2002), 成为类囊体膜第五大蛋白复合体。本文着重对近期模式植物蓝藻和拟南芥NDH在光合作用中的作用机理相关研究进展进行介绍, 对NDH参与光合作用的可能作用机制进行总结和展望。

1 叶绿体NDH复合体和蓝藻NDH-1复合体的组成与结构特征

三十年前, Ohyama等(1986)和Shinozaki等(1986)对烟草和苔藓两种质体全基因组进行测序发现, 质体中存在编码线粒体复合体I (complex I)亚基的11个*ndh*同源基因。后来, 通过生物信息学、蛋白质组学和遗传学等多种手段, 在叶绿体中又发现了17个核编码的*ndh*基因, 因此, 高等植物叶绿体NDH至少由28个NDH亚基组成。通过对叶绿体和蓝藻的基因组比对, 在蓝藻中发现15个高度同源的*ndh*基因, 即*ndhA~ndhO*, 也发现了相应的同源蛋白(Friedrich和Scheide 2000)。大肠杆菌NDH-1复合体是复合体I的最小组成模式, 由NuoA到NuoN 14个亚基组成, 这个最小的模式可以进行最基本的能量转化反应(Friedrich和Weiss 1997)。通过比较大肠杆菌、蓝藻、叶绿体的NDH组成,

收稿 2016-09-23 修定 2016-09-30

资助 国家自然科学基金(31470338)和国家重点基础研究发展计划("973"计划)项目(2015CB150104)。

* 通讯作者(E-mail: hlmi@sibs.ac.cn)。

就可以看出, NdhA~K是原核和真核生物NDH的保守成分(图1)。然而, 在叶绿体和蓝藻中都没有找到在大肠杆菌中参与NADH氧化的3个亚基NuoE、NuoF和NuoG的同源蛋白。由于这3个亚基含有NADH结合位点、辅基FMN以及铁硫簇, 类囊体膜NDH是否能直接氧化NADH或NADPH还有待于证实。目前认为, 叶绿体NDH的电子供体是铁氧还蛋白(ferredoxin, Fd), 而不是NAD(P)H (Shikanai 2016), 这是根据CRR31 (NdhS)亚基的C端具有类SH3 (Src homology)结构域, 具有Fd的结合位点, 加上体外实验证明还原型Fd能还原质醌等实验结果来推测的(Yamamoto等2011)。蓝藻的NdhS的C端是保守的, 说明NdhS是类囊体膜NDH电子接收的成分(Battchikova等2012)。我们实验室使用表面等离子共振(SPR)的方法, 证明了嗜热蓝藻Fd能与NdhS互作(He等2015)。近年来, 发现了另一个调节NDH活性的亚基——NdhV。拟南芥*NdhV*突变导致拟南芥NDH的不稳定和活性的下降(Fan等2015), 蓝藻*NdhV*突变导致集胞藻对高温敏感(Gao等2016), 丧失在高光下NDH活性的调节功能(Chen等2016)。

与叶绿体NDH复合体相比, 原核生物蓝藻的NDH-1复合体的组成与结构相对简单, 但形式多样。蓝藻NDH-1有三种不同形式: NDH-1L、NDH-1MS和NDH-1MS′, 分别参与呼吸、围绕光系统I的循环电子传递和CO_2吸收(Ogawa和Mi 2007)。陆生植物叶绿体NDH复合体的质体编码亚基和蓝藻参与呼吸和循环电子传递的NDH-1L组成高度相似, 甚至生理功能也很类似。由于叶绿体NDH、蓝藻NDH-1L与细菌的NDH-1具有相似性, 推测它们都呈L-型结构(图1)。

NdhA~NdhG是位于膜部分的亚基, 这7个亚基在原核细菌、蓝藻和叶绿体NDH中比较保守, 这些亚基的功能是维持NDH复合体的稳定, 以保持

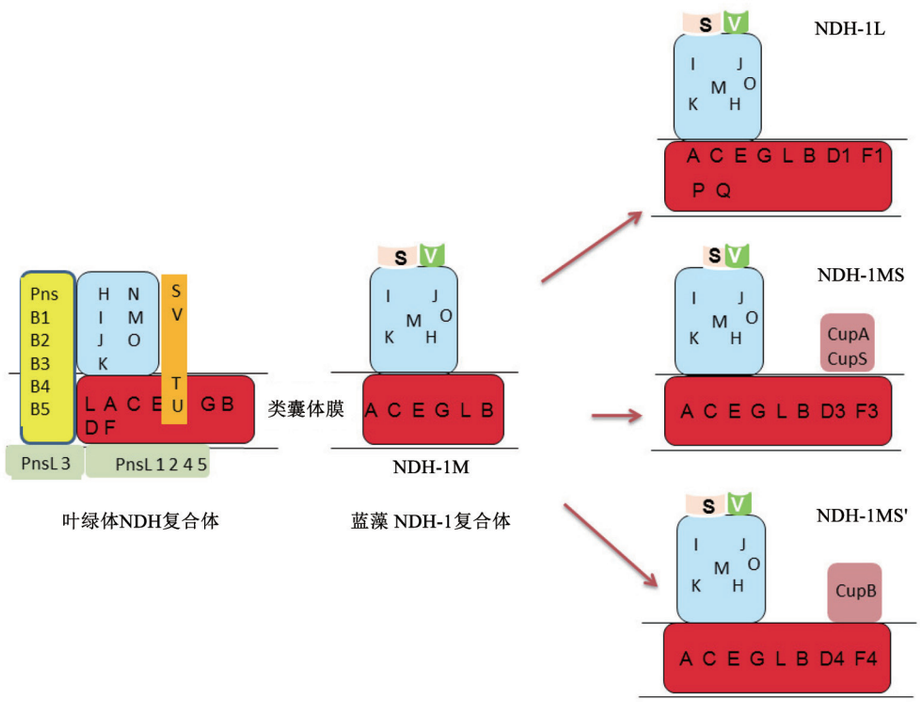

图1 叶绿体NDH和蓝藻NDH-1复合体模型图

Fig.1 Schematic models of chloroplast NDH and cyanobacterial NDH-1 complexes

叶绿体NDH复合体(左)根据Shikanai (2016)的模型绘制。蓝色部分为亚复合体A, 黄色部分为亚复合体B, 橘黄色部分为电子结合亚复合体, 红色部分为膜亚复合体, 绿色部分为稳定囊腔亚复合体。蓝藻NDH-1复合体(中和右)根据He和Mi (2016)的模型绘制。蓝色部分为亲水臂亚复合体, 红色部分为疏水亚复合体, S和V为电子接收亚基, 朱红色部分为CO_2吸收和转运亚基。NDH-1L参与呼吸和循环电子传递, NDH-1MS为低CO_2诱导型CO_2吸收复合体, NDH-1MS′为组成型CO_2吸收复合体。

其活性。蓝藻NdhB的缺失就会造成NDH-1复合体瓦解, 使NDH-1丧失活性(Zhang等2004)。缺失NdhB的拟南芥突变体, 整个NDH复合体几乎完全解聚(Hashimoto等2003)。

叶绿体NDH复合体含有更多的亚基, 已经在拟南芥中发现有组成亚复合体B的5个亚基(PnsB1~B5), 这部分与地钱的NDH是保守的, 亚复合体B的作用是维持整个植物NDH复合体的稳定性(Ueda等2012), PnsB3 (也被称为NDF4)具有铁流簇, 但似乎不参与从铁氧还蛋白(Fd)到PQ的电子传递, 因为在蓝藻中没有相应的亚基(Shikanai 2016)。蓝藻NdhP和NdhQ是从分离纯化嗜热蓝藻NDH-1L鉴定到的(Nowaczyk等2011), 它们的作用是稳定NDH-1L复合体(Schwarz等2013; Wulfhorst等2014; Zhang等2014; Zhao等2014)。拟南芥位于囊腔的亚复合体L由5个亚基(PnsL1~L5)组成两类蛋白, 这部分虽然与地钱没有保守性, 却能稳定NDH (Ifuku等2011)。第一类蛋白包括形成光系统II放氧复合体的蛋白, 如PnsL1是一个PsbP家族的成员(PPL2), 而PnsL2和PnsL3是PsbQ类似蛋白(Ishihara等2007; Suorsa等2010; Yabuta等2010)。虽然根据蛋白序列PnsL3被分类为亚复合体L, 但该蛋白对突变体NDH的稳定性实验表明, 它属于亚复合体B的成分(Suorsa等2010; Yabuta等2010)。PnsL4 (FKB16-2)和PnsL5 (CYP20-2)属于第二类成员, 是肽基脯氨基顺反异构酶(peptidyl-prolyl *cis-trans* isomerase) (Peng等2009; Sirpiö等2009), PnsL4、L5可能通过肽基脯氨基顺反异构酶参与蛋白折叠。NdhT (CRRJ)和NdhU (CRRL)可能是以异源二聚体的形式存在, 是NdhS和亚复合体A的积累所必需的(Yamamoto等2011)。叶绿体的NDH亚复合体L与光系统I互作而形成超复合体, PnsL1和PnsL3通过与Lhca5和Lhca6结合, 稳定NDH-光系统I超大复合体(Peng等2009)。

与线粒体复合体I相比, NdhL~O是放氧光合生物所特有的成分(Prommeanate等2004; Battchikova等2005; Rumeau等2005; Shimizu等2008)。NdhL参与蓝藻的CO_2浓缩功能(Ogawa 1991), 在高等植物中稳定NDH亚复合体A (Shimizu等2008)。NdhL~O都在稳定叶绿体亚复合体A中发挥作用(Peng等2009; Rumeau 2005)。通过功能蛋白组分析, 鉴定到集胞蓝藻的NdhN和NdhO (Prommeenate等2004; Battchikova等2005)。利用电子显微镜观察标记了荧光蛋白的NDH亚基的类囊体膜成分, 发现NdhL~NdhO是连在一起的(Birungi等2010)。最近, 我们通过研究集胞蓝藻不同的突变体和分析不同亚基之间互作, 发现NdhM位于蓝藻的NDH-1亲水臂的核心, 对NDH-1亲水臂的组装和活性起着关键的作用(He等2016)。蓝藻NdhN也影响到NDH-1亲水亚基的组装和活性(He和Mi 2016), 相比之下, NdhO虽然对NDH-1的活性的贡献不大, 但在无机碳受限的条件下, 在呼吸代谢中发挥重要的作用(He和Mi 2016)。

蓝藻NDH-1几种复合体都含有NDH-1M, 除了参与呼吸和围绕光系统I循环电子传递的NDH-1L以外, 还有参与CO_2吸收的NDH-1MS和NDH-1MS′复合体(图1)。NDH-1L是由NDH-1M与NdhD1或D2、NdhF1组成的, 而NDH-1MS由NDH-1M和NDH-1S (包含NdhD3、NdhF3、CupA、CupS)构成, NDH-1MS′则由NDH-1M和NDH-1S′ (包含NdhD4、NdhF4、CupB)构成。单粒子电子显微镜分析纯化后类囊体膜组分, 发现NDH-1MS呈U形结构(Arteni等2006), 其关键蛋白CupA结合于U形臂的先端(Folea等2008)。NDH-1MS对CO_2具有高亲和性, 并受低CO_2诱导。而NDH-1MS′是参与组成型CO_2吸收的复合体, 其关键基因*CupB*与*CupA*同源(Shibata等2001)。我们研究发现, CupB蛋白依赖于NdhD4定位于类囊体膜上, 并且通过分离纯化, 鉴定到1个450 kDa的NDH-1MS′复合体(Xu等2008)。

2 NDH复合体的生理功能

NDH作为光合膜蛋白第五大复合体介导蓝藻(Mi等1992, 1994, 1995)和高等植物围绕光系统I的循环电子传递(Shikanai等1998), 在调节光合作用中发挥重要的作用。图2是基于国际上系列研究工作, 对NDH的生理功能进行的归纳和总结。

2.1 为高效光合碳同化补充ATP

蓝藻之所以能够适应水中低二氧化碳浓度的环境, 是由于它们具有CO_2浓缩机制, 能够提高光合碳同化关键酶Rubisco活性部位周围的二氧化碳浓度、克服自身的Rubisco对CO_2的低亲和力, 从而有效地同化CO_2 (Price等1998; Kaplan和Reinhold

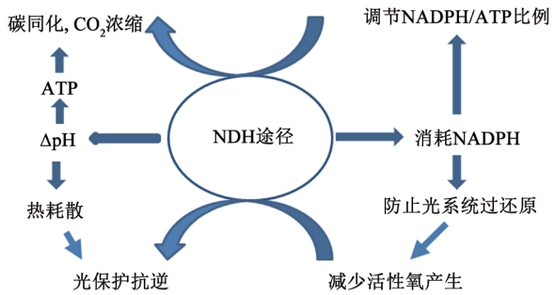

图2 NDH介导的围绕光系统I循环电子途径(NDH途径)的生理功能

Fig.2 Physiological functions of NDH-mediated cyclic electron flow around photosystem I

NDH消耗NADPH、经过NDH的电子传递耦联循环光合磷酸化, 产生额外ATP, 用于高效光合作用, 为不同时期调节NADPH/ATP比例; 同时, 依赖NDH的电子传递产生跨类囊体膜质子浓度差, 参与胁迫条件下的热耗散过程; 此外, NDH途径氧化NADPH, 防止光系统的过还原, 减少高温、高光胁迫条件下活性氧的产生, 参与了植物的光保护。

1999), 蓝藻的CO_2浓缩机制和无机碳运输都是耗用ATP的过程。完全丧失NDH活性的集胞藻PCC6803的*ndh*B缺失突变体(Ogawa 1990)、NdhH、J、N、M缺失突变体(He等2016; He和Mi 2016)不能够在空气CO_2的浓度中存活。丧失部分NDH活性的突变体在空气CO_2中比野生型生长缓慢(He和Mi 2016)。根据NDH-B缺失突变体丧失了NDH介导的围绕光系统I的循环电子传递(Mi等1992, 1995), Ogawa (1991, 1992)提出了NDH介导的循环电子传递为蓝藻二氧化碳浓缩机制和无机碳运输提供ATP。 Klughammer等(1999)利用聚球藻(*Synechococus* PCC7002)的*ndh*D3、*ndh*F3等突变体研究二氧化碳的吸收效率时, 观察到这些突变体既不能被诱导高效率的二氧化碳吸收, 也不能诱导高效率的碳酸根的运输, 认为由*ndh*D3和*ndh*F3编码的NDH特殊亚基参与高亲和性二氧化碳的吸收。Ohkawa等(1998)通过研究与二氧化碳浓缩机制有关的突变体的生长、无机碳的吸收等各种生理指标, 提出NDH参与能量传导和诱导高亲和性无机碳运输。

光化学反应中产生的ATP和NADPH作为同化力用于CO_2同化, 根据理论计算, 每同化1分子的CO_2所需要的ATP/NADPH的比值为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, 非循环磷酸化在产生2分子NADPH的同时最多只能产生2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变动环境时, 线性电子传递所产生固定比例的ATP和NADPH往往不能满足上述条件之下CO_2同化的需求, 由于循环电子传递途径消耗NADPH, 只产生ATP, 可以提供额外的ATP, 以满足光合碳同化对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, 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。我们在研究叶片高ATP含量的小麦优选后代10号时, 发现该株系具有较高的NDH含量和循环电子传递活性(He等2013)。相反, 在低光强条件下, 组装因子CRR6缺失而使NDH活性丧失的水稻突变体的碳同化效率低、引起生物量明显降低(Yamori等2015)。

Hibino等(1996)报道蓝藻*ndhK*和*ndhI*基因在高盐浓度下表达量增加, 同时NDH介导的光系统I循环电子传递也被促进。在高浓度盐的震激下, *ndhB*缺失突变体不能够恢复其光合作用能力, 因此认为NDH介导的围绕光系统I的循环电子传递是蓝藻适应盐胁迫所必需的(Tanaka等1997)。虽然, NDH在蓝藻适应盐胁迫的机理还不清楚, 根据植物适应胁迫环境是一个需要ATP的过程, 推测NDH介导的循环电子传递和叶绿体呼吸电子传递可能会提供更多的ATP以适应这些耗能的反应。

2.2 调节光合机构的运转

蓝藻和高等植物细胞内ATP与NADPH的需求及其形成的比例因光合环境或者发育阶段变动而异。例如当光呼吸发生时, 需要较高比例的ATP/NADPH (Hatch 1987)。当CO_2浓度变低时, 需要靠消耗能量来浓缩CO_2, 从而提高ATP的需求量, 非循环电子传递形成的ATP和NADPH的比例是一定的, 不易调节, 然而通过光合循环电子传递与呼吸电子传递的协同作用, 就使得调节ATP和NADPH

的比例成为可能。我们近两年发现, 水稻在开花灌浆期, 围绕光系统I的循环电子传递增强, 颖壳叶绿体中, 超复合体的含量和活性明显比剑叶的高, 同时, 循环电子传递耦联的跨膜质子梯度也明显地高。我们推测这是应灌浆期高ATP的需求而增强了NDH途径(Xu等2014)。我们利用叶绿体比较蛋白组学研究叶片具有高ATP含量的小麦优选后代时发现, 与亲本相比, 该株系中NDH途径的蛋白和ATP合酶的含量均比较高(He等2013), 因此, NDH介导的围绕光系统I的循环电子传递的主要作用是在特殊时期如开花灌浆期、环境胁迫条件下, 为有效地进行碳同化而调节ATP和NADPH的比例。

我们在研究光诱导的集胞藻PCC6803野生型和*ndh*B基因缺失突变体M55的NADPH荧光(蓝绿荧光)动力学也观察到, 在加入卡尔文循环的抑制剂碘乙酸(IAA)时, 野生型的光诱导的NAD(P)H荧光增加了2倍, 而突变体的却没有变化, 同时IAA抑制了基线以下的蓝绿荧光的下降。IAA诱导的光下NADPH的形成可以认为是卡尔文循环所消耗的NADPH。很明显, 野生型细胞中, 刚刚打开饱和作用光时, 卡尔文循环的活性很高, 可以防止NADP的完全还原。另一方面, 在NDH失活突变体中, NADP是处于完全还原的状态(Mi等2000)。因此, 我们认为在NDH失活突变体的细胞中, 由于缺失NDH介导的循环磷酸化, 导致ATP限制卡尔文循环的活性。

激发能在两个光系统之间的再分配(即状态转换)主要是对两个光系统的激发不平衡进行调节, 是植物抵御反应中心免受过多的激发能引起的光破坏的一种机制。在蓝藻中, Schreiber等(1995)报道了NDH通过介导围绕光系统I的循环电子传递参与激发能在两个光系统之间的再分配(状态转换), NDH失活的突变体丧失了状态转换的功能, 被锁定在状态1中。NDH介导的循环电子传递可能通过影响质醌的氧化还原状态而参与激发能的再分配(Ma等2007, 2008)。

植物通过调节循环电子传递或叶绿体呼吸电子传递来平衡其氧化还原系统, 使之不至于过度氧化或过度还原。循环电子传递和非循环电子传递的适当比例有可能达到这个目的。在正常生理条件下, 卡尔文循环充分活跃时, 主要是非循环电子流的电子还原质醌, 而循环电子传递的比例小到足以忽视。然而, 当CO_2的同化降低时, 就会造成NADPH和还原的铁氧还蛋白过多地积累, 循环电子传递就会被促进。同样, NDH介导的电子传递也在相应的条件下被启动和促进。当将暗适应的集胞藻细胞移到光下时, 诱导了NDH的表达并且促进了NDH介导的循环和呼吸电子传递(Mi等2001)。在低湿度的条件下, 烟草*ndh*B缺失突变体生长缓慢(Horvath等2000)。我们最近发现, NDH途径缺失后, 高温诱导的叶绿体呼吸途径的质体末端氧化酶(plastid terminal oxidase, PTOX)表达量的上调被显著地抑制, 从而使光合电子传递链处于过还原状态, 导致活性状态Rubisco活化酶下调, 最终导致光抑制(Li等2016)。因此, NDH介导的光系统I循环电子传递在各种逆境下调节包括叶绿体呼吸等途径, 以保证光保护作用的有效进行。

2.3 光破坏的防御作用

光合作用需要光能, 但过剩的激发能会引起光抑制, 甚至导致光系统II反应中心的破坏, 而围绕光系统I的循环电子传递参与将多余的光能通过热耗散的机制消除, 从而保护植物免受强光引起的光破坏损伤。围绕光系统I循环电子传递通过产生跨膜质子梯度对光系统II进行下游调节也可以减轻光抑制 (Heber和Walker 1992)。Endo等(1999)的研究发现烟草NDH失活突变体(*ndh*B缺失突变体)在饱和光下, 会产生严重的光抑制, 反复进行饱和光的照射后, 使得基质的过度还原而导致了系统II的光抑制导致叶绿素的漂白, 表明叶绿体NDH能够抵御强光引起的光抑制。Kofer等(1998)发现在缺失*ndh*C-J-K操纵子的烟草突变种的叶绿体中积累了大量的淀粉, 提出叶绿体呼吸在除去过多的光还原产物中的作用。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。Horvath等(2000)也观察到*ndh*B突变体在低湿下生长缓慢。我们发现烟草*ndh*C-J-K突变缺失会导致低温或高温胁迫下活性氧积累(Wang等2006)。此外, 低浓度$NaHSO_3$促进暗光转化条件下NDH介导的循环电子传递, 从而可以通过促进依赖NDH的循环光合磷酸化来减缓光氧化伤害, 最终提高光合作用(Wu等2011, 2012)。

3 结论与展望

NDH介导的围绕光系统I的循环电子传递在

植物不同的生育期、各种外界环境条件的胁迫条件下, 在维持有效的光合机构运转中发挥重要的作用。尽管目前对NDH的研究取得了很多重要的进展, 然而, NDH途径对光合机构高效运转的调控机理尚有待于阐明。仍然需要多方面的研究深入进行, 例如, NDH的活性域及电子供体尚有待于明确; 蓝藻不同的NDH-1复合体之间有什么联系? 它们是如何协同发挥调控作用的? NDH如何参与蓝藻和C_4植物CO_2浓缩机制等等。由于NDH含量少、体外不稳定、形式多样, 不易拿到晶体, 至今NDH的结构尚未得到解析。或许今后我们可以应用高分辨率的冷冻电镜, 对不同形式、不同状态的NDH进行观察和分析, 深入研究NDH的动态调控机制。随着分子生物学、蛋白质组学、代谢组学、计算生物学、结构生物学等新兴学科的发展、以及生物、物理、化学等多学科交叉渗透, 人们将可以揭示NDH复杂的调控网络, 这对于阐明光合作用高效传能转能的机理有着重要的意义。

参考文献

Arteni AA, Zhang P, Battchikova N, Ogawa T, Aro E-M, Boekema EJ (2006). Structural characterization of NDH-1 complexes of *Thermosynechococcus elongatus* by single particle electron microscopy. Biochim Biophys Acta, 1757 (11): 1469–1475

Battchikova N, Wei L, Du L, Bersanini L, Aro EM, Ma W (2012). Identification of novel Ssl0352 protein (NdhS), essential for efficient operation of cyclic electron transport around photosystem I, in NADPH: plastoquinone oxidoreductase (NDH-1) complexes of *Synechocystis* sp. PCC 6803. J Biol Chem, 286 (42): 36992–37001

Battchikova N, Zhang PP, Rudd S, Ogawa T, Aro EM (2005). Identification of NdhL and Ssl1690 (NdhO) in NDH-1L, and NDH-1M complexes of *Synechocystis* sp. PCC 6803. J Biol Chem, 280 (4): 2587–2595

Birungi M, Folea M, Battchikova N, Xu M, Mi HL, Ogawa T, Aro EM, Boekema EJ (2010). Possibilities of subunit localization with fluorescent protein tags and electron microscopy exemplified by a cyanobacterial NDH-1 study. Biochim Biophys Acta, 1797 (8): 1681–1686

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 (4): 868–876

Chen X, He Z, Xu M, Peng L, Mi H (2016). NdhV subunit regulates the activity of type-1 NAD(P)H dehydrogenase under high light conditions in cyanobacterium *Synechocystis* sp. PCC 6803. Sci Rep, 6: 28361

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 C4-type plant *Zea mays*. J Plant Physiol, 163 (8): 800–808

Endo T, Shikanai T, Takabayashi A, Asada K, Sato F (1999). The role of chloroplastic NAD(P)H dehydrogenase in photoprotection. FEBS Lett, 457 (1): 5–8

Fan X, Zhang J, Li W, Peng L (2015). The NdhV subunit is required to stabilize the chloroplast NADH dehydrogenase-like complex in *Arabidopsis*. Plant J, 82 (2): 221–231

Folea IM, Zhang P, Nowaczyk MM, Ogawa T, Aro EM, Boekema EJ (2008). Single particle analysis of thylakoid proteins from *Thermosynechococcus elongatus* and *Synechocystis* 6803: localization of the CupA subunit of NDH-1. FEBS Lett, 582 (2): 249–254

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 (1-2): 1–5

Friedrich T, Weiss H (1997). Modular evolution of the respiratory NADH: ubiquinone oxidoreductase and the origin of its modules. J Theor Biol, 187 (4): 529–540

Gao F, Zhao J, Wang X, Qin S, Wei L, Ma W (2016). NdhV is a subunit of NADPH dehydrogenase essential for cyclic electron transport in *Synechocystis* sp. strain PCC 6803. Plant Physiol, 170 (2): 752–760

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 (4): 541–549

Hatch MD (1987). C_4 photosynthesis: a unique blend of modified bichemistry, anatomy and ultrastructure. Biochim Biophy Acta, 895 (2): 81–106

He Z-H, Li H-W, Shen Y, Li Z-S, Mi H (2013). Comparative analysis of the chloroplast proteomes of a wheat (*Triticum aestivum* L.) single seed descent line and its parents. J Plant Physiol, 170 (13): 1139–1147

He ZH, Mi HL (2016). Functional characterization of the subunits N, H, J, and O of the NAD(P)H dehydrogenase complexes in *Synechocystis* sp. strain PCC 6803. Plant Physiol, 171 (2): 1320–1332

He Z, Xu M, Wu Y, Lü J, Fu P, Mi H (2016). NdhM is required for the stability and the function of NAD(P)H dehydrogenase complexes involved in CO_2 uptake in *Synechocystis* sp. strain PCC 6803. J Biol Chem, 291: 5902–5912

He Z, Zheng F, Wu Y, Li Q, Lv J, Fu P, Mi H (2015). NDH-1L interacts with ferredoxin via the subunit NdhS in *Thermosynechococcus elongatus*. Photosynth Res, 126 (2-3): 341–349

Heber U, Walker D (1992). Concerning a dual function of coupled cyclic electron-transport in leaves. Plant Physiol, 100 (4): 1621–1626

Hibino T, Lee BH, Rai AK, Ishikawa H, Kojima H, Tawada M, Shimoyama H, Takabe T (1996). Salt enhances photosystem I content and cyclic electron flow via NAD(P)H dehydrogenase in the halotolerant cyanobacterium *Aphanothece halophytica*. Aust J Plant Physiol, 23 (3): 321–330

Horvath 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 (4): 1337–1349

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 (9): 1560–1568

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

Kaplan A, Reinhold L (1999). CO_2 concentrating mechanisms in photosynthetic microorganisms. Annu Rev Plant Physiol Plant Mol Biol, 50: 539–570

Klughammer B, Sultemeyer D, Badger MR, Price GD (1999). The involvement of NAD(P)H dehydrogenase subunits, NdhD3 and NdhF3, in high-affinity CO_2 uptake in *Synechococcus* sp. PCC7002 gives evidence for multiple NDH-1 complexes with specific roles in cyanobacteria. Mol Microb, 32 (6): 1305–1315

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 (1-2): 166–173

Kubicki A, Funk E, Westhoff P, Steinmuller 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 (2): 276–281

Li Q, Yao Z-J, Mi H (2016). Alleviation of photoinhibition by co-ordination of chlororespiration and cyclic electron flow mediated by NDH under heat stressed condition in tobacco. Front Plant Sci, 7: 285

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 (1): 221–233

Ma W, Deng Y, Mi H (2008). Redox of plastoquinone pool regulates the expression and activity of NADPH dehydrogenase supercomplex in *Synechocystis* sp. strain PCC 6803. Curr Microbiol, 56 (2): 189–193

Ma W, Ogawa T, Shen Y, Mi H (2007). Changes in cyclic and respiratory electron transport by the movement of phycobilisomes in the cyanobacterium *Synechocystis* sp. strain PCC 6803. Biochim Biophy Acta, 1767 (6): 742–749

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 (2): 163–173

Mi HL, Deng Y, Tanaka Y, Hibino T, Takabe T (2001). Photo-induction of an NADPH dehydrogenase which functions as a mediator of electron transport to the intersystem chain in the cyanobacterium *Synechocystis* PCC6803. Photosynth Res, 70 (2): 167–173

Mi HL, 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 (4): 661–668

Mi HL, Endo T, Schreiber U, Ogawa T, Asada K (1992). 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 (8): 1233–1237

Mi HL, Klughammer C, Schreiber U (2000). Light-induced dynamic changes of NADPH fluorescence in *Synechocystis* PCC 6803 and its *ndh*B-defective mutant M55. Plant Cell Physiol, 41 (10): 1129–1135

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 (3): 361–371

Nowaczyk MM, Wulfhorst H, Ryan CM, Souda P, Zhang H, Cramer WA, Whitelegge JP (2011). NdhP and NdhQ: two novel small subunits of the cyanobacterial NDH-1 complex. Biochemistry, 50 (7): 1121–1124

Ogawa T (1990). Mutants of *Synechocystis* PCC 6803 defective in inorganic carbon transport. Plant Physiol, 94: 760–765

Ogawa T (1991). A gene homologous to the subunit-2 gene of NADH dehydrogenase is essential to inorganic carbon transport of *Synechocystis* PCC6803. Proc Natl Acad Sci USA, 88 (10): 4275–4279

Ogawa T (1992). Identification and characterization of the *ictA/ndhL* gene product essential to inorganic carbon transport of *Synechocystis* PCC6803. Plant Physiol, 99 (4): 1604–1608.

Ogawa T, Mi H (2007). Cyanobacterial NADPH dehydrogenase complexes. Photosynth Res, 93 (1-3): 69–77

Ohkawa H, Sonoda M, Katoh H, Ogawa T (1998). The use of mutants in the analysis of the CO_2-concentrating mechanism in cyanobacteria. Can J Bot, 76 (6): 1035–1042

Ohyama K, Fukuzawa H, Kohchi T, Shirai H, Sano T, Sano S, Umesono K, Shiki Y, Takeuchi M, Chang Z, et al (1986). Chloroplast gene organization deduced from complete sequence of liverwort marchantia-polymorpha chloroplast DNA. Nature, 322 (6079): 572–574

Peng LW, 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 (11): 3623–3640

Price GD, Sultemeyer D, Klughammer B, Ludwig M, Badger MR (1998). The functioning of the CO_2 concentrating mechanism in several cyanobacterial strains: a review of general physiological characteristics, genes, proteins, and recent advances. Can J Bot, 76 (6): 973–1002

Prommeenate P, Lennon AM, Markert C, Hippler M, Nixon PJ (2004). Subunit composition of NDH-1 complexes of *Synechocystis* sp PCC 6803 – Identification of two new *ndh* gene products with

nuclear-encoded homologues in the chloroplast Ndh complex. J Biol Chem, 279 (27): 28165–28173

Rumeau D, Becuwe-Linka N, Beyly A, Louwagie M, Garin J, Peltier G (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 (1): 219–232

Schreiber U, Endo T, Mi HL, Asada K (1995). Quenching analysis of chlorophyll fluorescence by the saturation pulse method: particular aspects relating to the study of eukaryotic algae and cyanobacteria. Plant Cell Physiol, 36 (5): 873–882

Schwarz D, Schubert H, Georg J, Hess WR, Hagemann M (2013). The gene *sml0013* of *Synechocystis* species strain PCC 6803 encodes for a novel subunit of the NAD(P)H oxidoreductase or complex I that is ubiquitously distributed among Cyanobacteria. Plant Physiol, 163 (3): 1191–1202

Shibata M, Ohkawa H, Kaneko T, Fukuzawa H, Tabata S, Kaplan A, Ogawa T (2001). Distinct constitutive and low-CO_2-induced CO_2 uptake systems in cyanobacteria: genes involved and their phylogenetic relationship with homologous genes in other organisms. Proc Natl Acad Sci USA, 98 (20): 11789–11794

Shikanai T (2016). Chloroplast NDH: a different enzyme with a structure similar to that of respiratory NADH dehydrogenase. Biochim Biophys Acta, 1857 (7): 1015–1022

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 (16): 9705–9709

Shimizu H, Peng L, Myouga F, Motohashi R, Shinozaki K, Shikanai T (2008). CRR23/NdhL is a subunit of the chloroplast NAD(P)H dehydrogenase complex in *Arabidopsis*. Plant Cell Physiol, 49 (5): 835–842

Shinozaki K, Ohme M, Tanaka M, Wakasugi T, Hayashida N, Matsubayashi T, Zaita N, Chunwongse J, Obokata J, Yamaguchishinozaki K, et al (1986). The complete nucleotide sequence of the tobacco chloroplast genome: its gene organization and expression. EMBO J, 5 (9): 2043–2049

Sirpio S, Allahverdiyeva Y, Holmstrom M, Khrouchtchova A, Haldrup A, Battchikova N, Aro EM (2009). Novel nuclear-encoded subunits of the chloroplast NAD(P)H dehydrogenase complex. J Biol Chem, 284 (2): 905–912

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 (6): 877–883

Tanaka Y, Katada S, Ishikawa H, Ogawa T, Takabe T (1997). Electron flow front NAD(P)H dehydrogenase to photosystem I is required for adaptation to salt shock in the cyanobacterium *Synechocystis* sp. PCC 6803. Plant Cell Physiol, 38 (12): 1311–1318

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 (4): 683–693

Wang P, Duan W, Takabayashi A, Endo T, Shikanai T, Ye JY, Mi HL (2006). Chloroplastic NAD(P)H dehydrogenase in tobacco leaves functions in alleviation of oxidative damage caused by temperature stress. Plant Physiol, 141 (2): 465–474

Wu Y, He W, Ma W, Shen Y, Mi H (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 (30): 3872–3877

Wu Y, Zheng F, Ma W, Han Z, Gu Q, Shen Y, Mi H (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 (10): 1734–1743

Wulfhorst H, Franken LE, Wessinghage T, Boekema EJ, Nowaczyk MM (2014). The 5 kDa protein NdhP is essential for stable NDH-1L assembly in *Thermosynechococcus elongatus*. PLoS One, 9 (8): e103584

Xu M, Ogawa T, Pakrasi HB, Mi HL (2008). Identification and localization of the CupB protein involved in constitutive CO_2 uptake in the cyanobacterium, *Synechocystis* sp strain PCC 6803. Plant Cell Physiol, 49 (6): 994–997

Xu M, Shi N, Li Q, Mi H (2014). An active supercomplex of NADPH dehydrogenase mediated cyclic electron flow around Photosystem I from the panicle chloroplast of *Oryza sativa*. Acta Biochim Biophy Sin, 46 (9): 757–765

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 (6): 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 (4): 1480–1493

Yamori W, Shikanai T, Makino A (2015). Photosystem I cyclic electron flow via chloroplast NADH dehydrogenase-like complex performs a physiological role for photosynthesis at low light. Sci Rep, 5: 13908

Zhang J, Gao F, Zhao J, Ogawa T, Wang Q, Ma W (2014). NdhP is an exclusive subunit of large complex of NADPH dehydrogenase essential to stabilize the complex in *Synechocystis* sp. strain PCC 6803. J Biol Chem, 289 (27): 18770–18781

Zhang PP, Battchikova N, Jansen T, Appel J, Ogawa T, Aro EM (2004). Expression and functional roles of the two distinct NDH-1 complexes and the carbon acquisition complex NdhD3/NdhF3/CupA/Sll1735 in *Synechocystis* sp. PCC 6803. Plant Cell, 16 (12): 3326–3340

Zhao J, Rong W, Gao F, Ogawa T, Ma W (2015). Subunit Q is required to stabilize the large complex of NADPH dehydrogenase in *Synechocystis* sp. strain PCC 6803. Plant Physiol, 168 (2): 443–451

The regulation of NAD(P)H dehydrogenase complexes bound in thylakoid membranes in photosynthesis

MI Hua-Ling*

Laboratory of Photosynthesis and Environmental Biology, National Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China

Abstract: In photosynthesis, photoreaction is efficiently driven by protein complexes bound in the thylakoid membrane. In addition to photosystem I, photosystem II, cytochrome *b6/f* complex, and ATPase, another complex, NAD(P)H dehydrogenase (NDH) complex mediated cyclic electron transports involved in protection of plants against environmental stressed for efficient photosynthesis becomes 5th protein complex in the thylakoid membrane. So far, there are many researches on the components, the assembly and the physiology function of NDH. However, the regulative mechanism of NDH in photosynthesis is still remained to be clarified. In this review, the progress on the research of NDH is introduced. Finally, the regulative role of NDH in photosynthesis is summarized and the future research on NDH is discussed.

Key words: NAD(P)H dehydrogenase complex; cyclic electron flow; photosynthesis; chloroplast; cyanobacteria

Received 2016-09-23 Accepted 2016-09-30

This work was supported by the National Natural Science Foundation of China (Grant No. 31470338) and National Key Basic Research Program of China ("973" Program) (Grant No. 2015CB150104).

*Corresponding author (E-mail: hlmi@sibs.ac.cn).