植物光合作用循环电子传递的研究进展

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摘要: 在植物光合作用的光反应中, 由叶绿素等色素分子收集的光能通过光系统I (PSI)和光系统II (PSII)驱动了光合电子传递进程。当电子最终被传递到高能化合物NADPH和ATP中, 完成光能到化学能的转换并为固碳反应提供能量的过程, 被称为线形电子流(LEF)。而电子仅绕着PSI传递, 通过形成跨膜质子梯度而驱动ATP酶合成ATP的过程, 被称为循环电子流(CEF)。近年来, CEF的催化机制、生理作用和调控机制等吸引了研究者们的关注, 特别是反向遗传学的发展, 为更深入理解循环电子传递带来契机。本文综述了CEF的研究历史和最新进展, 并从植物逆境适应以及植物进化等角度进一步分析了CEF的形成与生理意义, 为今后的相关研究提供参考。

关键词: 循环电子流; 线形电子流; 光合作用; 光合磷酸化

1 光合作用中的电子传递

光合作用是植物利用光能, 合成有机物并释放氧气(O_2)的过程。人们将光合作用的多个主要化学反应步骤分为两部分: (1)将光能转化为生物所能直接利用的化学能, 即形成还原型烟酰胺腺嘌呤二核苷磷酸(nicotinamide adenine dinucleotide phosphate, NADPH)并合成三磷酸腺苷(adenosine triphosphate, ATP); (2)将ATP和NADPH中储存的能量进一步转化, 固定二氧化碳(CO_2)以形成单糖与多糖。第一部分反应需要直接的光照提供能量, 称为光反应; 而第二部分则间接地利用光反应形成的ATP和NADPH作为能量源泉, 反应本身不需要直接光照提供能量, 称为暗反应。由光反应产生的比例合适的ATP/NADPH分子是暗反应顺利进行的基础。因此, 叶绿体还拥有一套复杂系统, 在不同环境条件下对ATP与NADPH的产量进行精密调控。

叶绿体类囊体膜是光反应的发生场所, 光系统II (PSII)、细胞色素*b*$_6$*f*复合体(Cyt *b*$_6$*f*)、光系统I (PSI)和ATP合酶(ATPase)等多种蛋白复合物均定位于类囊体膜上。在放氧复合体中通过裂解水分子产生的电子依次经过PSII、Cyt *b*$_6$*f*和PSI, 最后传递给烟酰胺腺嘌呤二核苷磷酸(nicotinamide adenine dinucleotide phosphate, NADP)形成NADPH。此过程中产生的跨膜质子梯度驱动ATP合酶, 形成ATP。这样的电子传递流称为线形电子流(linear electron flow, LEF), 与之耦联的ATP形成过程则被称为非循环式光合磷酸化(noncyclic photophosphoryation, NCPSP)。若电子经PSI后没有传递给NADP, 而是经Cyt *b*$_6$*f*或质体醌(plastoquinone, PQ)又回到PSI, 传递过程中仅形成跨膜质子梯度, 合成ATP。这样围绕PSI进行传递的电子传递方式被称为循环电子流(cyclic electron flow, CEF), 与CEF相耦联的ATP合成过程则被称为循环式光合磷酸化(cyclic photophosphoryation, CPSP)。相比较而言, LEF途径需PSI与PSII均参与其中, 电子传递过程同时形成NADPH和ATP两种高能化合物; 而CEF过程只有PSI参与, 产生跨膜质子梯度并通过ATP酶合成ATP, 电子并不参与形成NADPH (图1)。由此可见, PSI对电子在LEF或CEF传递途径的选择中起重要作用, 也是电子传递过程中形成CEF的关键节点。而电子在光合电子传递过程中所经过的途径和受体分子决定了ATP与NADPH的产量。

2 循环电子传递途径

CEF现象最初由Arnon等人在离体菠菜(*Spinacia oleracea*)叶绿体中发现(Arnon等1954), 他们于1958年明确提出了循环光合磷酸化和非循环光合磷酸化的概念(Arnon等1958)。国内最早报道CEF是在1964年, 中国科学院上海生命科学研究院植物生理生态研究所(原中国科学院上海植物生理研究所)观察到甘薯(*Dioscorea esculenta*)离体叶细胞制剂内的CEF (沈巩楙等1964)。也是在上世纪60年代, 人们开始初步描述构成CEF途径的生化反应历程。美国加利福尼亚大学Tagawa等第一次提出铁氧还蛋白(ferredoxin, Fd)可以作为一个电子载体调控CEF, 这个途径也被称为Fd依赖途径

收稿 2016-11-01 **修定** 2017-02-10

资助 中央高校基础研究基金(JC2013-2)和国家自然科学基金(31271433)。

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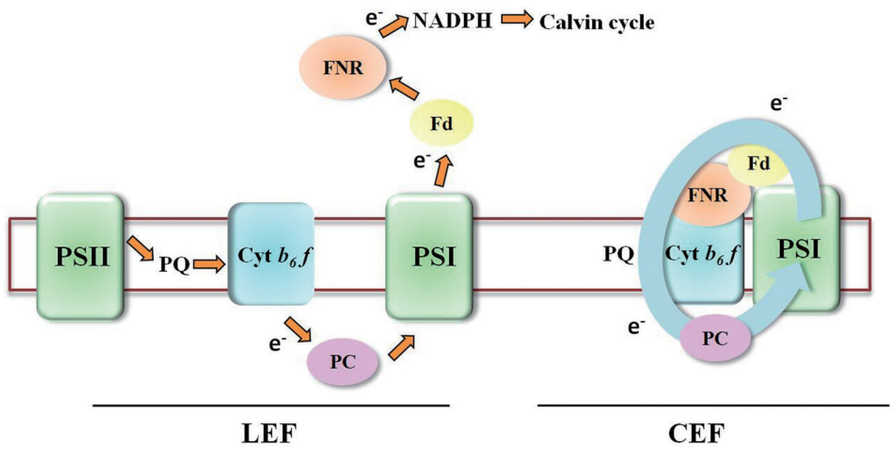

图1 LEF和CEF示意图

Fig.1 Schematic representation of LEF and CEF

根据Iwai等(2010)文献修改。LEF: 线形电子流; CEF: 循环电子流; Cyt b_6f: 细胞色素b_6f复合体; e⁻: 电子; Fd: 铁氧还蛋白; FNR: Fd-NADP氧化还原酶; NADPH: 还原型烟酰胺腺嘌呤二核苷磷酸; PC: 质体蓝素; PQ: 质体醌; PSI: 光系统I; PSII: 光系统II。

(Fd-dependent CEF), 该途径受抗霉素A (antimycin A, AA)抑制(Tagawa等1963)。几十年过后, 日本京都大学的研究者提出了CEF途径的另一个调控蛋白, 即由NAD(P)H脱氢酶复合体(NAD(P)H dehydrogenase complex, NDH)调控的NDH依赖途径(NDH-dependent CEF) (Mi等1992a)。

2.1 FQR途径(PGR5/PGRL1介导途径)

对电子传递途径的研究, 不仅需要对途径中的多个电子载体清晰描述, 也需要对催化电子传递途径的酶进行深入研究。早在1984年, 就有研究者提出证据, 认为有一种特别的酶参与对AA敏感的CEF传递途径, 并将其命名为铁氧还蛋白-质体醌-还原酶(ferredoxin-plastoquinone-reductase, FQR) (Moss和Bendall 1984)。他们认为该传递路径为: PSI→Fd→FQR→PQ→Cyt b_6f (图2-A) (Bendall和Manasse 1995; Cleland和Bendall 1992)。然而, 当时并没有分离出FQR蛋白, 因此之后围绕FQR的研究和争论持续了很多年。

2002年, Munekage等首次确认了PGR5 (proton gradient regulation 5)蛋白是拟南芥(*Arabidopsis thaliana*) CEF过程中一个必不可少的组分, PGR5是一个类囊体小蛋白, 参与CEF电子传递过程(Hertle等2013), 其表达量提高可使CEF增强(Okegawa等2008); 反之, 在室外条件下生长的拟南芥突

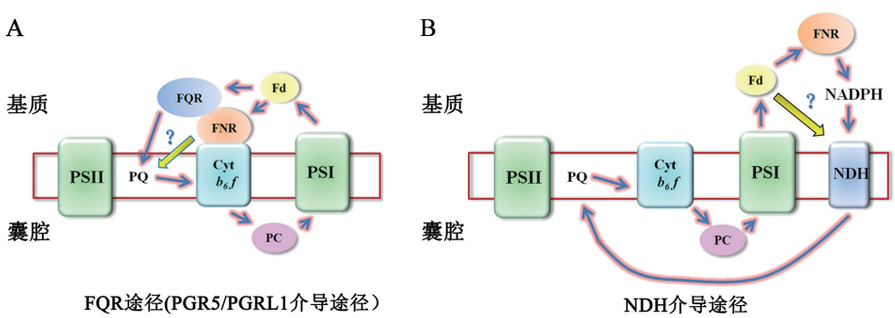

图2 高等植物CEF的两种传递路径图示

Fig.2 Schematic representation of two major routes in PSI-driven CEF in high plants

根据Joliot等(2006)和Shikanai (2007)文献修改。FQR: 铁氧还蛋白-质体醌-还原酶; NDH: NAD(P)H脱氢酶复合体; Cyt b_6f: 细胞色素b_6f复合体; Fd: 铁氧还蛋白; FNR: Fd-NADP氧化还原酶; NADPH: 还原型烟酰胺腺嘌呤二核苷磷酸; PC: 质体蓝素; PQ: 质体醌; PSI: 光系统I; PSII: 光系统II。

变体*pgr5*, 与野生型比较, 死亡率更高(Munekage等2002)。2008年, PGRL1 (proton gradient regulation like 1)又被确认为拟南芥CEF过程中另一个主要成分, PGRL1具有两个跨膜螺旋结构, 可以将电子从Fd转移到PQ (DalCorso等2008; Hertle等2013)。PGRL1与PGR5均能与Fd互作, PGRL1/PGR5对诱导热耗散(nonphotochemical quenching, NPQ)和保护PSII免受光抑制是必需的(DalCorso等2008)。在真核生物中, 电子从Fd传递到PGRL1需要PGR5蛋白介导, 这种依赖于PGR5的电子传递调控方式是植物在适应光强快速变化时保护PSI免受光伤害的关键。而如果缺少这两个蛋白其中之一, CEF都会受损, 因此研究者们认为PGR5和PGRL1蛋白可能是FQR的组成成分(DalCorso等2008; Iwai等2010; Munekage等2008; Shikanai 2007; Suorsa等2012)。到2013年, Hertle等(2013)发表文章称PGRL1很可能就是大家寻找了多年的神秘蛋白FQR。

当研究者们尝试研究FQR的进化历程时还发现, PGR5和PGRL1并非同时起源的一组功能蛋白。在蓝藻植物集胞藻(*Cyanobacterium synechocystis* sp. PCC6803)基因组中, 只找到*PGR5*同源基因而缺失与高等植物*PGRL1*同源的基因(Yeremenko等2005)。莱茵衣藻(*Chlamydomonas reinhardtii*)的情况却恰恰相反, 其基因组中只有*PGRL1*, 却没有*PGR5*, 但存在另外一种团藻目特有蛋白PETO (Volvocales-specific protein)。故而推测蓝藻中可能存在着其他CEF途径, 而莱茵衣藻中则由PETO代替PGR5作为CEF超级复合体中调控光合系统状态转换的因子之一(Iwai等2010)。

另有文献报道, 高等植物中, 电子也可以由Fd通过Fd-NADP氧化还原酶(Fd-NADP oxidoreductase, FNR)绑定在Cyt b_6f复合体的Q_i位点, 被传递至Cyt b_6f复合体或者再回到PQ的循环电子传递途径: PSI→Fd→FNR→PQ (图2-A), 并且有生物化学或者分子生物学的手段证实了FNR确实参与了CEF (Shahak等1981; Joliot和Johnson 2011; Böhme 1977), FNR可以作为Fd绑定位点绑定在类囊体膜上(Tic62和TROL为其绑定蛋白), 也可能会从Fd得到电子后直接还原PQ池(Bojko等2003) (图2-A)。除此之外, FNR还参与另一条CEF途径, 即NDH依赖途径(Quiles和Cuello 1998)。

2.2 NDH介导途径

NDH是线粒体呼吸电子传递链上的原初电子传递体, 它可以将电子从还原型烟酰胺腺嘌呤二核苷酸(reduced form of nicotinamide-adenine dinucleotid, NADH)传给辅酶Q (ubiquinone)。有趣的是, 随着烟草(*Nicotiana tabacum*)和地钱(*Marchantia polymorpha*)叶绿体基因组测序完成, 人们在叶绿体中首次发现了NDH复合体的同源编码基因(Ohyama等1986; Shinozaki等1986)。叶绿体NDH复合体被认为起源于蓝藻中的NDH-1复合体(Friedrich和Weiss 1997), 由11个叶绿体基因编码(*ndhA~ndhK*), 通常绑定在类囊体膜上, 通过Lhca5和Lhca6与PSI形成超级复合体(Peng等2011), NDH是NDH-途径CEF的关键调控蛋白(Sazanov等1996; Endo等1998; Shikanai等1998; Peng和Shikanai 2011)。电子流通过NDH复合体, 被传递到PQ, 但在此电子传递链中, NDH的电子供体一直存有争议(Shikanai 2007)。在高等植物离体叶绿体中, 当Fd存在时, NADPH可以提供电子使之进入光合系统间的传递(Munekage等2004; Okegawa等2008)。因此, NADPH可能是CEF传递过程中NDH的电子供体(PSI→Fd→FNR→NADPH→NDH→PQ→Cyt b_6f)。但近期的文献认为NDH最可能直接从还原态的Fd处得到电子(图2-B) (Leister和Shikanai 2013; Peng等2011; Yamamoto和Shikanai 2013; Yamori和Shikanai 2016)。

根据Johnson提出的模型, CEF传递过程中, 还原态的Fd除了供给$NADP^+$电子外, 还可以通过FQR或者NDH给PQ池提供电子(Johnson 2011)。然而, NDH在类囊体膜内含量很低, 不能够胜任快速的CEF过程(>100 s^{-1}) (Joliot等2004; Joliot和Joliot 2002)。因此, NDH依赖途径只在植物的CEF传递过程中占有较小份额, 主要的CEF传递还是以PGR5/PGRL1途径为主(Shikanai 2007)。

而当我们从植物演化的角度来审视NDH-CEF时, 会进一步发现CEF电子传递的复杂性。低等植物除了轮藻纲(Charophyceae)和绿枝藻纲(Prasinophyceae)的一些物种外, 其他包括莱茵衣藻在内的很多绿藻甚至裸子植物种中的一些重要种群, 例如买麻藤纲(Gnetopsida)和松柏纲(Coniferopisda)多数植物的叶绿体基因组中缺失了整套*nd-*

hA~*K*基因, 但它们仍然具有CEF的能力(Braukmann等2009; Wakasugi等1994; Maul等2002; Martín和Sabater 2010), 因此, 在这些植物中, 肯定存在另一种CEF途径代替NDH途径。例如, 有人提出在衣藻的CEF传递过程中PQ的还原是通过另一种类型的NADH: PQ氧化还原酶(a type II NADH: PQ oxidoreductase, Nda2)来实现(Desplats等2009)。Nda2结构简单, 不泵出质子, 与NDH相比, 催化CEF反应要快2倍。因此, Nda2可能是一条更容易掌控的能量平衡途径(Kramer和Evans 2011)。

在蓝藻中, NDH-1复合体既能调控CEF, 也可以调控叶绿体呼吸电子传递链(Ogawa和Mi 2007; Mi等1992a, 1992b, 1994)。同样, 在高等植物中分离出的NDH复合体对NADH显示出底物专一性, 在黑暗中, NDH很可能会利用由淀粉分解产生的NADH来还原PQ池, 因此, 有研究者认为NDH在高等植物中可能是黑暗条件下叶绿体的呼吸复合体(Sazanov等1998; Tikhonov 2013)。另外, NDH-CEF虽然比例小, 但对植物能起重要保护作用, 高温胁迫下, NDH复合体可以通过调控CO_2同化来优化光合电子传递链从而减少活性氧物质对植物的毒害(Wang等2006)。最近3年又有研究发现, 在弱光下, NDH也可以显著调控CEF途径的活性, 并在暗胁迫条件下起重要作用(Yamori等2015; Tikhonov 2013)。

3 CEF的生理作用

3.1 维持ATP/NADPH平衡

对于光合作用的暗反应, 即固碳作用而言, 维持合适的ATP/NADPH比例至关重要, 国内研究者于1961年在世界范围内最早测得植物内ATP/NADPH比值(殷宏章等1961)。通常植物体内1个分子CO_2同化成碳水化合物至少需要3个ATP和2个NADPH, 即ATP/NADPH比值为1.5 (Bendall和Manasse 1995; Breyton等2006; Munekage等2004; Johnson 2011)。而植物中非循环光合磷酸化合成ATP/NADPH的最高比值一般为1.3, 这部分ATP差额由CEF传递过程合成(Allen 2003; Cruz等2005; Eberhard等2008; Foyer等2012; Joliot等2006; Kramer等2004; Seelert等2000; 沈允钢等2010)。在CEF途径中, NADPH不是电子的最终归宿, 围绕PSI的电子循环能有利于形成跨越类囊体膜的质子梯度, 通过ATP酶合成ATP以维持ATP和NADPH的平衡。一直以来, 人们低估了CEF的生理作用, 近期研究表明, CEF对提高ATP/NADPH比例和保护光合系统免受因叶绿体过度还原而造成的伤害具有必不可少的作用(Yamori和Shikanai 2016)。

3.2 保护光系统, 提高植物环境适应性

通常认为CEF是一种可供植物选择的电子传递途径, 它不仅可以驱动ATP合成酶合成ATP, 加强热耗散, 保护PSII, 稳定和保护放氧复合体, 还可以保护PSI。因此, CEF被认为是植物本身具有的一种十分重要的光保护机制。在干旱、强光、弱光、高温和低温等逆境胁迫下, 植物可以激发CEF来适应环境胁迫。但是, 至今仍不清楚CEF在逆境胁迫下是如何响应并启动调控机制。

在干旱胁迫条件或者CO_2限制(固碳活动受限制)时, CEF被激活, 提供ATP, 限制PSII单线态氧形成, 产生质子梯度, 诱导热耗散, 防止光合系统的光氧化, 从而保护PSI并对PSII系统进行修复, 帮助植物抵御逆境(Canaani等1989; Heber和Walker 1992; Munekage等2002; Golding和Johnson 2003)。水分胁迫也会促进CEF的激发(Nandha等2007)。例如, 生长在潮汐带的大型藻类石莼(*Ulva* sp.)在正常生理条件下, PSI驱动的CEF和LEF比起来要弱得多, 但在退潮的干旱周期内, 却可以检测到很强的CEF, 也因此提供了ATP, 对光合器官起到重要的保护作用, 使得石莼可以存活。并且在重新复水的过程中, PSI比PSII活性恢复快。因此, PSI驱动的CEF是石莼可以在每天的涨潮和退潮中克服干旱条件而正常存活的原因之一(Gao等2011)。中国科学院水生生物所王强课题组还进一步发现: 在缺N条件下, 小球藻(*Chlorella sorokiniana* C3)和衣藻中CEF速率和ATP合成酶活性显著增强, 而衣藻*pgrl1*突变体在缺N条件下CEF速率下降, 中性脂类积累较少。进一步分析发现, 在绿藻中, Ca^{2+}可调控PGRL1途径CEF, 从而提供ATP以诱导缺N条件下脂类的生物合成(Chen等2015)。

轻微高温胁迫会提高ATP的需求量, 导致NADPH/ATP比例偏高和PQ库的非光化学还原, 进而激活NDH-CEF, 使得植物抵抗高温(Wang等2006)。CEF可以产生高质子梯度, 使类囊体腔酸化, 引发热耗散, 从而在强光下保护PSII不会因过

度还原而造成伤害(Makino等2002)。通过基粒区(appressed region)天线蛋白发生热耗散, 防止PSII和PSI受到光抑制(Miyake 2010; Munekage等2004; Shikanai 2007; Yamori等2015)。例如, 烟草在强光条件下CEF的激发会伴随热耗散增强(Miyake等2005); 拟南芥*pgr5*突变体的PSI对强光非常敏感, 在强光下热耗散受到抑制, 而野生型拟南芥的PSI对强光不敏感, 这表明CEF在高光下的激发可以维持高热耗散, 从而保护光系统(Munekage等2002, 2004)。

在低温条件下, CEF对PSII也能起到重要的光保护作用, 例如, 植物因短时间低温胁迫受损的PSII在弱光条件下可以因CEF激发而快速恢复(Clarke和Johnson 2001; Bukhov和Carpentier 2004; Hirotsu等2005; Huang等2011)。而在长时间的低光照和低温生长条件下(20°C), 正常水稻PSI的相对电子传递速率(electron transportation rate, ETR)和CO_2同化率也均高于突变体水稻, NDH-途径CEF在低温和低光照下对水稻光合作用和生长起重要作用(Yamori等2011, 2015)。

另外, 国内学者在研究CEF的基础上还提出了改善光合磷酸化合成ATP能力的途径, 中国科学院上海生命科学研究院沈允钢课题组早在上世纪七八十年代就发现喷洒低浓度的亚硫酸氢钠($NaHSO_3$)可以提高作物叶片的光合速率(魏家绵等1989), 增强CEF及其相耦联的光合磷酸化作用(Ben等2005)。之后, 中国科学院上海生命科学研究院米华玲课题组提出低浓度的$NaHSO_3$可以同时增强烟草的LEF和CEF过程, 调控NDH介导的CEF, 提高光合磷酸化形成的ATP合成速率, 提高光合放氧速率, 减轻光氧化胁迫, 增强植物光合作用(Wu等2011, 2012)。而在拟南芥中, 低浓度的$NaHSO_3$可以同时增强NDH复合体介导的循环电子传递和PGR5/PGRL1介导的循环电子传递活性, 并且对后者增强效果更显著(李娜等2016)。

3.3 CO_2浓缩

蓝藻和C_4植物在进化过中具有一种特殊的环境适应机制, 即CO_2浓缩机制(CO_2 concentrating mechanism, CCM)。CCM可以显著提高光合效率, 尤其对适应低CO_2环境具有非常重要的意义(Maeda等2002; Price 2011; Takabayashi等2005)。蓝藻能够通过CCM提高光合碳同化关键酶——二磷酸核酮糖羧化酶(ribulose bisphosphate carboxylase oxygenase, Rubisco)活性部位周围的CO_2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 (米华玲2016)。在蓝藻植物*Synechococcus* PCC7942和PCC7002中, 有2种与NDH-1复合体相关的CO_2水合作用蛋白(CO_2 hydration proteins)——ChpX (NDH-I_4复合体)和ChpY (NDH-I_3复合体)与CO_2摄取活动有关(Maeda等2002; Woodger等2007)。ChpX和ChpY可调控CO_2水合为HCO_3^-, 并和NDH-1复合体一起与电子传递和质子跨膜转移相关联(Maeda等2002; Price 2011)。C_4植物在维管束鞘细胞中可以最大限度地浓缩CO_2, 比C_3植物固碳效率高, 但与C_3植物相比, 每固定1个CO_2, C_4植物却需要多使用2个ATP, 但LEF因其产生固定的ATP/NADPH而不能单独额外提供ATP (Kramer和Evans 2011), 因此, 这部分差额ATP由CEF产生(Takabayashi等2005; Kramer和Evans 2011; Ishikawa等2016)。在C_4植物中, NDH-CEF是主要途径, NDH也成为C_4植物光合作用时维管束鞘细胞中启动CCM的重要蛋白, 而FQR-途径(PGR5/PGRL1) CEF的贡献很小(Takabayashi等2005)。

4 LEF与CEF转换

光合电子传递链的调控是影响植物健康生长和存活的关键。LEF和CEF途径的电子分配在植物适应环境变化过程中起重要作用。LEF和CEF途径转换是一个复杂的过程, 受到多种因素的调控。通常情况下, 当LEF受到抑制时, CEF发挥作用, 产生跨类囊体膜质子梯度, 合成ATP用于修复PSII的核心蛋白D_1。当PSI活性受到严重破坏, PSII的修复就会受阻, 整个光合作用系统崩溃(Huang等2010a)。在PSI活性保持稳定的前提下, PSII损伤的修复速度很快, 通常在几个小时就可以完成(Huang等2010b)。

4.1 电子传递链上电子载体的竞争

CEF和LEF共享许多电子载体, 例如: PQ、Cyt b_6f、质体蓝素(plastocyanin, PC)、PSI、Fd和FNR, 它们存在着潜在的竞争关系(Fork和Herbert 1993; Bendall和Manasse 1995)。LEF和CEF的交替与电子传递的氧化还原状态有关, 因此, 调节两种电子传递流的机制之一是二者之间还原当量的竞争,

而Fd和FNR位于光合电子传递的最末端, 对电子的去向起关键作用。

Hald等(2008a, b)提出, 竞争还原态的Fd可能是PSI受体侧电子流发生分枝的关键点。Fd的含量决定了CEF的运行, Fd过表达的植物其CEF传递能力也增加(Yamamoto等2006)。在特殊条件下, 当PSI受体侧被过度还原时, CEF传递过程会显著优先于LEF。例如, 暗适应的叶绿体, 卡尔文循环中消耗NADPH缓慢, 就会将电子从LEF转移至CEF (Joliot和Joliot 2005), 从而产生ΔpH和ATP。同时, 当光诱导卡尔文循环和LEF增加时, CEF则减少。因此, 植物在稳定的状态下, LEF会优于CEF (Breyton等2006; Joliot和Joliot 2005; Kuvykin等2011; Trubitsin等2005)。

此外, PQ库的氧化还原状态对CEF也有调节作用。PQ氧化发生在Cyt b_6f复合体的Q_o位点, 伴随有质子释放, 和其他生理过程(大约1 ms)比起来, PQ氧化(1~20 ms)要慢得多, 因此PQ氧化也被认为是LEF传递过程中的限速反应(Yamori和Shikanai 2016)。当PQ库处于半还原状态时, CEF达到最大值; 反之, 当PQ库被完全还原或者完全氧化时, CEF不能被激活(Allen 2003)。

另外, 在光下, 卡尔文循环激活可以加快LEF, 但当卡尔文循环受限时, CEF会增加(Miyake 2010), 因CO_2固定受限使得NADPH/ATP的比例升高, 通过反馈调节会导致电子传递链(Cyt b_6f、PC和P700)的氧化以及PQ库的还原, 这时处于还原态的PSI电子受体如Fd和NADPH会将电子传递给那些电子传递链上的电子载体, 形成CEF。

可溶性的FNR是多种氧化还原路径交叉点的关键酶, 其在膜与基质中的分布位置可以调控电子流, 进一步影响LEF和CEF过程(Benz等2010)。FNR可以与多种类囊体蛋白例如PsaE (Andersen等1992)、Cyt b_6f (Zhang等2001)、NDH (Guedeney等1996)和PGRL1 (DalCorso等2008)互作, 从而参与LEF和CEF过程。在LEF中, FNR没有绑定Cyt b_6f, 电子从Fd被传递到NADP, 而在CEF中, FNR与Cyt b_6f的结合为Fd提供了一个绑定位点, 使电子传递至膜上的Q_i位点(Joliot和Johnson 2011)。

4.2 光合系统状态转换

LEF和CEF转换的另一个机制是状态转换(state transitions) (Finazzi等2002, 1999)。在莱茵衣藻中, 电子传递在LEF和CEF之间的转换取决于两个光合系统的激发平衡, 当PSII处于优先激发态时, PQ和Cyt b_6f被还原, LHCII发生磷酸化, 与PSII分离, 迁移至PSI, 因此从PSII向PSI重新分配激发能(状态II, CEF占优势); 当PSI处于优先激发态时, LHCII-P去磷酸化, 重新迁移回PSII, 此时从PSI向PSII重新分配激发能(状态I, LEF占优势) (Finazzi等2002)。但也有研究报道, 莱茵衣藻中的CEF现象可以不受状态转换影响(Takahashi等2013)。

在拟南芥中也发现与状态转换相关的激酶STN7, 在PSII优先激发时, STN7可以使LHCII磷酸化, 从PSII分离之后迁移(Tikkanen等2010)。*stn7*突变体不能在波动光下生长, 因此, STN7可能与平衡光照有关, 有助于应对ATP/NADPH改变(Kramer和Evans 2011)。而结合*pgr*和*stn7*突变体的表型差异来看, 拟南芥中CEF现象与状态转换并不相关(Munekage等2002; Bellafiore等2005; Yamori和Shikanai 2016)。

4.3 超级蛋白复合体

还有一些研究者认为CEF过程中可能存在着一种超级复合体, 包括Cyt b_6f、PC、PSI、Fd和FNR (Joliot等2004; Joliot和Joliot 2002, 2005)。在蓝藻中, 尽管连接NDH-1与PSI的蛋白与高等植物中的不同, 但也存在NDH-1-PSI超级复合物, 该超级复合体仅仅参与循环电子传递, 与呼吸电子传递无关(Gao等2016)。在衣藻中, Wollman和Bulté (1989)首先分离出含PSI和Cyt b_6f的复合体。之后Iwai等(2010)在莱茵衣藻中成功分离纯化出PSI-LHCI-LHCII-FNR-Cyt b_6f-PGRL1超级复合体, 并且认为这种超级复合体的形成和分解不仅控制着两个光合系统之间的能量平衡, 而且还可以转换光合电子流模式。在高等植物中, Clark等(1984)和Zhang等(2001)分别在菠菜和层理边枝藻(*Mastigocladus laminosus*)中分离和纯化出了含FNR和Cyt b_6f蛋白的复合物。而在拟南芥中, 只证实PGRL1-PGR5复合体可以与PSI互作, 从而促进拟南芥CEF超级复合体的形成(Breyton等2006; DalCorso等2008), 该潜在的CEF超级复合体尚未被完整分离。

但在拟南芥中, Peng等(2009)分离出了NDH-PSI超级复合体, 同时提出Lhca5和Lhca6这两个光

捕获蛋白对稳定完整NDH-PSI超级复合体结构有重要作用。在水稻颖壳叶绿体中, 中国科学院上海生命科学研究所米华玲课题组也分离鉴定出了NDH超级复合体, 这个高活性的超级复合体使NADPH-依赖的NDH途径CEF高度活化, 在水稻开花到灌浆期, 可以产生更大的跨膜质子梯度, 增加PSII最大光合效率, 对水稻更高效进行光合作用形成籽粒和光保护方面至关重要(Xu等2014)。

光诱导的类囊体膜重组也影响LEF和CEF之间的转换(Vallon等1991)。Cyt b_6f复合体在基质和基粒片层都有分布, 基粒片层分布的b_6f只参与LEF过程, 基质片层的b_6f则同时参与LEF和CEF (Albertsson 2001)。在状态I和状态II转换过程中, 随着磷酸化的LHCII向PSI横向迁移, 相当一部分的b_6f复合体从基粒膜向基质膜迁移, 这将会诱导超级复合体PSI-LHCI-LHCII-b_6f-FNR-PGRL1形成, 参与CEF传递(Vallon等1991)。也有人提出这种超级复合体的形成和分解可能会转换光合电子传递链的模式, 从而调控PSI和PSII之间的能量平衡。同时, 随着LHCII和b_6f蛋白复合体在富含PSII的基粒膜区向富含PSI的基质膜区迁移, 光合膜结构发生变化, 也解除了基质内电子载体被过度还原的危险(Iwai等2010)。

5 植物进化过程中的CEF

5.1 藻类植物

CEF在蓝细菌(蓝藻) (Carpentier等1984)及其他藻类(Maxwell和Biggins 1976; Ravenel等1994)中都发挥重要生理作用。在蓝藻基因组中, 虽然找到*PGR5*同源基因(Yeremenko等2005), 但PGR5-CEF很微弱(而该途径在高等植物叶绿体CEF中占优势主导地位)。蓝藻中没有真正的叶绿体, 主要通过NDH-1复合体进行CEF途径(Ogawa和Mi 2007), 后来逐渐进化的陆生植物叶绿体NDH中则出现了新的亚复合体并与PSI结合成超级复合体, 从而促进NDH更高效作用(Peng等2009)。在莱茵衣藻中, CEF电子流可以达到总电子流的50% (Forti等2003), CEF提供额外的ATP可以帮助藻类植物抵御盐胁迫和干旱胁迫而正常存活(Jeanjean等1993; Gao等2011)。对莱茵衣藻*pgr5*突变体和维管植物的相似突变体进行研究后发现, PGR5对两者的贡献是一致的, 因此PGR5是莱茵衣藻的CEF活性必不可少的组分(Johnson等2014)。

5.2 C_3植物

C_3植物虽然具备进行CEF的能力, 但CEF是否在稳态光合作用条件下, 即PSII正常工作时进行一直存有争议。最初的报道认为C_3植物稳态光合作用中并不存在CEF, 对C_3植物来说无关紧要(Herbert等1990), 但后续的研究认为CEF对光合作用贡献了大量电子流, 至少在特定的条件下如应对干旱、高光照、缺氧和低CO_2等环境胁迫时起重要作用(Clarke和Johnson 2001; Joliot和Joliot 2002; Golding和Johnson 2003; Golding等2004; Rumeau等2007; Joët等2002)。近期研究显示, 即使C_3植物在正常无胁迫时, CEF对调节ATP/NADPH比例来说也是必不可少的(Wang等2015; Yamori和Shikanai 2016)。例如Munekage等(2004)提出, 缺乏CEF的拟南芥突变体生长发育会受到严重损伤。

在正常生长条件下的C_3植物中, CEF大概只有LEF的5%~15% (Cruz等2005; Livingston等2010; Eberhard等2008; Kuvykin等2011; Kohzuma等2009), 或者由于LEF能够满足植物叶绿体代谢的ATP需要, 或是其他过程, 例如水-水循环(water-water cycle)和苹果酸盐阀(malate valve)已经足够平衡ATP/NADPH, CEF几乎检测不到。然而, 即使是低比例的CEF也可以形成ΔpH, 合成ATP, 维持叶绿体内ATP/NADPH比例平衡。目前, 在豌豆(*Pisum sativum*)和大麦(*Hordeum vulgare*)类囊体膜中已纯化出NDH蛋白(Sazanov等1998; Quiles等2000), 在烟草*ndh*突变体中, 用叶绿素荧光参数动力学和抑制剂阻断分析也已经证实NDH复合体参与C_3植物体内由PSI驱动的CEF过程(Joët等2001)。而PGR5-PGRL1途径是C_3植物拟南芥中主要的CEF途径(Munekage等2002, 2004), 在拟南芥中, PGR5和PGRL1可能与PSI、Cyt b_6f、FNR以及Fd形成超级复合体, 但至今尚未分离出该超级复合体(DalCorso等2008)。

5.3 C_4植物

CEF在C_4植物的特定组织内可以发挥显著作用(Asada等1993; Herbert等1990)。植物C_3-C_4进化过程中, NDH蛋白表达量显著增加(Munekage等2010; Nakamura等2013), 在C_4植物中, NDH-CEF起着至关重要的作用(Ishikawa等2016)。而对C_3植物

来说, 除胁迫条件外, NDH-CEF很少起生理效应(Ishikawa等2008; Yamori等2011)。C_4植物的维管束鞘细胞中很少存在PSII, ATP几乎完全由CEF提供(Woo等1970; Leegood等1981), NDH是C_4植物中最主要的CEF途径调控蛋白。加利福尼亚大学的Majeran等人(2008)研究发现*ndh*基因在玉米的维管束鞘中大量表达, 马里兰大学的Takabayashi等(2005)也提出, 在不同的C_4植物中, 与PGR5相比, NDH的表达与CEF途径更相关。但日本奈良先端科学技术大学院大学的Munekage等人(2010)却发现在黄菊属(*Flaveria*)中一些C_4植物和一些C_3-C_4过渡植物的维管束鞘细胞中, NDH和PGR5都有大量分布。在这些过渡种里, 也有人研究发现只要NDH在维管束鞘细胞内表达上调, 其他所有类型细胞内的PGR5和PGRL1都会跟着上调(Nakamura等2013), 因此认为在C_4植物的光合作用中, 两种途径CEF可能都存在。总之, 相对于C_3植物而言, 某些C_4植物种内NDH途径CEF增强, 而另一些C_4植物种内则是PGR5-PGRL1途径CEF增强(Ishikawa等2016)。

5.4 裸子植物

以往的报道没有针对裸子植物CEF的研究, 本实验室以裸子植物杉木(*Cunninghamia lanceolata*)为试验材料, 发现在完全黑暗状态下萌发的杉木幼苗子叶可以合成叶绿素且发育出具有发达基质和基粒片层结构的黄化叶绿体(etiochloroplast), PSI的电子传递效率高于PSII的电子传递效率。而且在完全黑暗条件下萌发的杉木子叶中, NDH-H和FNR蛋白的表达量相对于LEF光合电子传递链相关蛋白如PsaC、D1、CF1α的表达量更为丰富。重要的是, 在黑暗下生长的幼苗中, FNR蛋白主要结合在Cyt b_6f复合体上; 而在光照下的幼苗中, FNR蛋白则多以游离态存在。这说明杉木在完全黑暗培养状态下, FNR蛋白可能和Cyt b_6f蛋白结合, 从而赋予黄化叶绿体能接收光能并通过CEF进行光能转化的潜力(Xue等2017), 显示出裸子植物幼苗在出土后可能具有一种与常见模式被子植物不同的暗形态建成到光形态建成的转化机制, 然而, 这样的假说还需要更多验证。

6 CEF测量方法

CEF现象被发现报道已有50多年的历史, 随着反向遗传学的不断发展, 研究者们可以利用各类突变体(包括藻类植物、拟南芥、烟草、水稻、玉米等植物)来对CEF进行更深入研究, 但在植物体内定量分析CEF一直都是难点和争论点。CEF定量的难点在于无法将那些额外通过PSI的电子流与CEF准确区分开从而计算CEF的净产额(Shikanai 2014)。而且, CEF对各电子载体的氧化还原平衡和跨膜质子梯度高度敏感(Heber等1978; Allen 2003; Kramer和Evans 2011), 任何改变氧化还原平衡和质子梯度的实验方法都可能会影响到CEF。因此, 现在还没有哪一种测量CEF的方法是完全准确可靠的, 但通过多种CEF测定方法的综合应用, 也能为研究提供坚实依据(Fan等2016)。这些方法可粗略分为直接法和间接法两大类。直接方法包括通过ATP的合成(Bendall和Manasse 1995; Allen 2003)、Fd的氧化还原变化(Cleland和Bendall 1992)、叶绿素荧光的增加(Endo等1998; Munekage等2002)、$P700^+$的再还原(Harbinson和Woodward 1987; Schreiber 1988; Maxwell和Biggins 1976)、远红光下P700的氧化动力学(Joliot和Joliot 2002, 2005; Nandha等2007; Vredenberg和Bulychev 2010)、Cyt b_6f的氧化还原状态(Lam和Malkin 1982)、光声光谱学技术(Herbert等1990; Ravenel等1994)、电致变色信号(Baker等2007; Joliot和Joliot 2008)等方法来估量CEF活性(Fan等2016); 间接方法可以通过测量ETR1和ETR2来估量CEF (Fan等2016)。这些都为以后进一步开展CEF研究工作提供借鉴。

7 总结与展望

尽管CEF提供的能量和LEF比起来相对较低, 但CEF对叶绿体内的能量微调和氧化还原平衡起重要作用, 同时, 对绿藻固碳、C_4植物光合作用以及C_3植物适应环境胁迫具有重要的作用。目前, CEF还有很多未解决的问题, 在今后的研究中, 结合各种研究手段, 系统地研究光合电子流不同途径分配比例和不同电子传递途径在保护光合机构中的作用, 对深入阐明植物光合作用光防御和探讨提高光能利用效率的途径具有重要的意义。

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Research progress of cyclic electron transport in plant photosynthesis

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Abstract: In light reaction of plant photosynthesis, the light energy collected by chlorophylls drives the electron transport process via photosystem I (PSI) and II (PSII). When electrons are transferred to the high energy compounds, NADPH and ATP, all received light energy is completely transferred. This pathway supplies the reaction substrates for the carbon reaction and is called linear electron flow (LEF). When electrons only flow around the PSI, creating a proton gradient across the membrane, the pathway of the electron transport is named cyclic electron flow (CEF). During the CEF, ATP is the only products by driving the ATPase. In recent years, CEF has attracted researchers' attention, studies about the catalytic mechanisms, physiological effects and regulatory mechanisms have been raised. Importantly, the development of reverse genetics also brought opportunities to deep understanding about CEF. In this article, we review the study history and the latest progress of CEF. Furthermore, we analyze the activation and physiological effects of CEF from the aspects of stress adaption and evolution, providing a collection of references for further researches.

Key words: cyclic electron flow; linear electron flow; photosynthesis; photophosphorylation

Received 2016-11-01 Accepted 2017-02-10

This work was supported by the Fundamental Research Funds for the Central Universities (Grant No. JC2013-2) and the National Natural Science Foundation of China (Grant No. 31271433).

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