植物非光依赖叶绿素合成研究进展

薛娴[1,*], 万迎朗[2], 石欣隆[1], 曾繁梓[1]

[1]河南科技大学农学院, 河南洛阳471000

[2]海南大学热带农林学院, 海口570228

摘要: 植物叶绿素暗合成能力与非光依赖型原叶绿素酸酯还原酶(DPOR)有关, 光合生物进化过程中从细菌到裸子植物均保留了这个古老的酶, 完全黑暗条件下可以合成叶绿素。被子植物则由于丢失了这个酶, 失去了叶绿素暗合成能力。本文综合国内外有关非光依赖叶绿素合成的研究成果, 从叶绿素合成途径过程的关键反应、叶片中质体结构及光系统发育等方面, 对黑暗条件下植物叶绿素合成进行综述, 并从植物进化角度对叶绿素暗合成及其影响因素做了总结, 为研究植物暗形态建成以及暗-光转变过程中光合器官建成提供基础。

关键词: 叶绿素暗合成; 非光依赖型原叶绿素酸酯还原酶; 光系统发育

光是影响植物叶绿素合成和叶绿体发育的重要环境因子, 被子植物幼苗在黑暗条件下不能合成叶绿素而呈现黄化状态。与被子植物截然不同的是, 某些植物类群如藻类、苔藓、蕨类和裸子植物具有叶绿素暗合成的能力。这种现象和非光依赖型原叶绿素酸酯还原酶(light-independent protochlorophyllide reductase, DPOR)有关。DPOR在完全黑暗条件下可催化原叶绿素酸酯(Pchlide)还原, 使叶绿素合成顺利进行。

1 植物非光依赖叶绿素合成途径

植物叶绿素合成途径从谷氨酰t-RNA到叶绿素*a*和*b*的所有反应及相关基因都已被确认(Fujita和Yamakawa 2017), 以往文献对叶绿素暗合成途径的研究多集中于2个反应: δ-氨基酮戊酸(δ-aminolevulinic acid, ALA)合成反应和Pchilide还原反应。

1.1 ALA合成反应

ALA合成是被子植物叶绿素合成途径的第一步反应, 也是关键限速反应。该反应由谷氨酰-tRNA还原酶(glutamyl-tRNA-reductase, GluTR, *HEMA*编码)催化。光照可以显著诱导被子植物ALA合成(Kruse等1995; Mohanty等2006); 但在能进行叶绿素暗合成的植物类群中, 光照并不能显著促进GluTR和ALA合成。例如, 黑暗和光照条件下生长的莱茵衣藻(*Chlamydomonas reinhardtii*)中GluTR合成能力相近(Nogaj和Beale 2005; Nogaj等2005); 欧洲黑松(*Pinus nigra*)和欧洲云杉(*Picea abies*)幼苗ALA合成量在黑暗和光照条件下也基本相同(Demko等2009; Drazic和Bogdanovic 2000; Stolárik等2017)。

光照条件下, 被子植物添加外源ALA可增加Pchlide和叶绿素含量(Awad 2008; Memon等2009)。黑暗条件下, 被子植物添加外源ALA可以促进Pchlide合成(Castelfranco等1974; Granick 1970)。裸子植物欧洲云杉是松科植物中叶绿素暗合成能力最高的物种(Fujita和Bauer 2003), 黑暗中添加ALA也可以显著提高叶绿素含量(Pavlovič等2009); 而给黑暗中生长的美国蓝叶松(*P. jeffreyi*)、欧洲黑松和欧洲落叶松(*Larix decidua*)施用ALA后并没有起到类似效果, 虽然提高了Pchlide合成量, 但幼苗中叶绿素含量仍很低(Dražić和Mihailović 1998; Maximová和Slováková 2014; Michel-Wolwertz和Bronchart 1974)。这可能由于叶绿素暗合成中起关键作用的DPOR通常只与天然ALA结合, 添加外源ALA后, 大量Pchlide结合光依赖型原叶绿素酸酯还原酶(light-dependent protochlorophyllide reductase, LPOR, 又称POR), 抑制了DPOR合成, 继而影响叶绿素合成(Michel-Wolwertz和Bronchart 1974)。

黑暗条件下, 被子植物Pchide结合LPOR会影响ALA合成, FLU-like与CHL27蛋白复合体的存在也会负调控GluTR从而抑制ALA合成(Apitz等2016;

收稿 2019-09-25 **修定** 2020-06-19

资助 河南科技大学博士启动基金(13480079)和河南科技大学实验技术开发基金(1819019)。

* 通讯作者(xuexian2011@126.com)。

Kauss等2012)。但在裸子植物子叶中, ALA合成不受以上因素影响, 可以持续合成(Demko等2010)。因此, ALA合成不是裸子植物叶绿素暗合成的限速反应(Maximová和Slováková 2014)。裸子植物中可能存在2个独立的ALA库, 分别为同时存在的2种催化酶LPOR和DPOR提供底物(Stolárik等2017)。

1.2 Pchlide还原反应

Pchlide还原反应是被子植物叶绿素合成途径的另一关键限速反应。除厌氧光合细菌和被子植物外, 其他植物光合有机体中该反应均可通过2条途径进行: 光依赖型(光合成)和非光依赖型(暗合成)合成途径。光合成途径由LPOR催化, 在NADPH参与下, 在光下催化Pchlide还原; 暗合成途径由DPOR催化, 完全黑暗条件下即可进行(图1) (Armstrong 1998; Gabruk和Mysliwa-Kurdziel 2015; Reinbothe等2010; Schoefs和Franck 2003)。

LPOR和DPOR虽然催化同一反应, 但二者的编码基因、蛋白结构以及催化机制完全不同(Gabruk等2012)。LPOR为单体酶, 由核基因*POR*编码; DPOR由3个多肽组成, 分别由3个叶绿体基因即*ChlL*、*ChlN*和*ChlB* (光合细菌为*BchL*、*BchN*和*BchB*)编码(Muraki等2010; Nomata等2014; Sarma等2008)。被子植物中, 叶绿体和核基因组内均未发现*ChlLNB*同源物(Maximová和Slováková 2014; Reinbothe等2010)。

1.2.1 LPOR (POR)

LPOR首次从黄化叶片中成功分离至今已60多

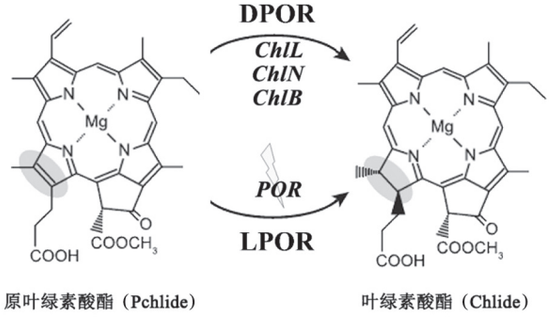

图1 Pchlide还原反应的两条途径

Fig.1 The two pathways of Pchlide reduction

本图引自Yamamoto等(2017)文献稍作修改。图中阴影部分显示被LPOR和DPOR催化的C_{17}=C_{18}的D环双键。

年(Smith和Kupke 1956)。LPOR是自然界已发现的3种光驱动酶之一(Aubert等2000; Sorigué 2017; Zhang等2019), 在光下需NADPH作为还原剂催化反应(Silva 2014)。不同光下, LPOR催化效率有差别, 红光(647 nm)下催化效率较高, 是蓝光(407 nm)下的3~7倍(Hanf等2012)。系统进化分析显示, LPOR首先出现在蓝藻中, 对氧不敏感。LPOR与SDR (short-chain dehydrogenase-reductase)家族序列高度相似, 是植物响应大气成分演化(无氧到有氧进化)的结果(Yang和Cheng 2004)。

被子植物中, LPOR是唯一催化Pchlide还原反应的酶。由于水平基因转移(horizontal gene transfer, HGT)和基因组复制, LPOR进化过程中出现了不同的亚型, 藻类出现了3个分支: 鞭毛藻类(dinoflagellates)、绿蜘藻类(chlorarachniophytes)和不等鞭毛藻类(stramenopiles)。被子植物拟南芥(*Arabidopsis thaliana*)经历了3次全基因组复制事件, 形成3个亚型: PORA、PORB和PORC。到目前为止, 拟南芥是唯一已确认具有3个亚型的物种(Gabruk和Mysliwa-Kurdziel 2015; Oosawa等2000; Su等2001)。PORA和PORB在植物早期黄化阶段大量合成, PORB和PORC在稍成熟幼苗及后续成熟植株中大量合成。见光后, PORB和PORC的含量与叶绿素*a*含量及类囊体的垛叠有直接关系(Masuda等2003; Frick等2003)。

*PORA*和*PORB*受EIN3 (ethylene insensitive 3)和EIL1 (EIN3-like 1)正调控(Zhong等2009)。除N末端转运肽不同外, PORA和PORB结构在所有植物中高度一致, 但二者功能不同, 不能彼此替代。PORA只在黄化幼苗初始见光阶段起作用, PORB在植物光适应和脱黄化过程中均起作用。光是*PORA*的强负调控因子, 见光后, *PORA*表达量迅速下降, 而*PORB*仍能持续表达。裸子植物中, *PORA*和*PORB*的表达量不仅与光有关, 还与植物组织部位和发育阶段有关。例如, 火炬松(*P. taeda*)子叶和幼嫩初生针叶(2个月苗)中, *PORA*转录和蛋白水平在见光前后差异显著, 见光后, *PORA*显著下降(含量低, 可以检测到), *PORB*无显著差异; 而在成熟的次生针叶(2年生苗)中, 只检测到*PORB*, *PORA*消失(Skinner和Timko 1999)。

*PORC*在黑暗中受PIF3 (phytochrome interacting factor 3)、HDA1 (histone deacetylase 1)和SCLs (scarecrow-like proteins)抑制, 通常表达量极低或不表达。而PIF1 (phytochrome interacting factor 1)则通过结合*PORC*启动子的G-box DNA序列, 在黑暗中正调控*PORC*表达。见光后, PIF3磷酸化而失活, 组蛋白H4乙酰化, 同时, 光照促进*miR171*表达, *miR171*抑制*SCLs*转录, 因此, *PORC*表达量显著上调。此外, 转录因子HY5 (long hypocotyl 5)也可以促进*PORC*表达, *HY5*在黑暗中受COP1/SPA1 (constitutive photomorphogenic 1/suppressor of phytochrome A 1)复合体阻断, 见光后, COP1/SPA1与PhyB互作, 释放HY5转录因子, *PORC*表达量上调(图2)。

乙烯信号转导途径调控*PORA*和*PORB*, COP1和PIF1则调控*PORC*, 这几个调控机制之间也相互影响。光照下应用乙烯后, EIN3调控COP1从细胞质转移至细胞核, 阻断COP活性, 抑制*PORC*表达; 无乙烯存在时, COP1则主要定位于细胞质, *HY5*则启动*PORC*转录(图2)。EIN3/EIL1与PIF1互作还可以阻止幼苗免受光氧化伤害, 促进子叶变绿(Zhong等2009)。

LPOR为核编码蛋白, 需通过其N末端转运肽转运至质体。研究该转运现象所用试验材料通常为大麦(*Hordeum vulgare*) (只有PORA和PORB)。PORA可能是目前确认的唯一一个转入细胞器需

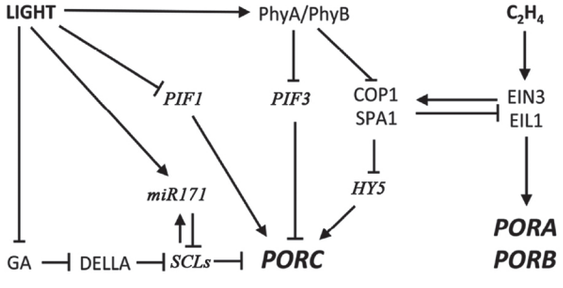

图2 拟南芥*PORA*、*PORB*和*PORC*基因表达调控图

Fig.2 Scheme of the network regulating the expression of *PORA*, *PORB* and *PORC* in *Arabidopsis*

本图引自Gabruk和Mysliwa-Kurdziel (2015)一文。图中总结了拟南芥中调控LPOR表达的两大因子(光照和乙烯)。箭头表示正调控, 钝端线条表示负调控。

要依赖底物的酶, 其转运肽的5个氨基酸基序(TTSPG)对结合Pchlide至关重要。PORA的底物依赖特性只在拟南芥子叶中存在, 在真叶中, PORA输入质体不再需要底物(Kim和Apel 2004)。PORB输入质体则不需要结合底物(Gabruk和Mysliwa-Kurdziel 2015; Plöscher等2009; Reinbothe等2008)。

黑暗下形成的白色体(etioplast)中, LPOR处于失活状态, 常与Pchlide以及NADPH形成高度规则的原(前)片层体(prolamellar bodies, PLBs)晶格聚合结构(POR:Pchlide:NADPH=1:1:1) (Forreiter等1991; Solymosi和Schoefs 2010)。近年, 蓝藻POR-Pchlide-NADPH三元复合体结构已被成功解析(Zhang等2019)。三元复合体见光后, Pchlide进行光转化, 叶绿素开始合成, 晶格结构分解, 基粒开始堆叠, 叶绿体形成(Denev等2005; Gabruk和Mysliwa-Kurdziel 2015; Masuda等2003; Solymosi等2007)。从外部形态上表现为子叶变绿并打开, 下胚轴弯钩伸展, 开始光形态建成过程(Forreiter等1991; McNellis和Deng 1995)。

1.2.2 DPOR

DPOR是一个很古老的酶, 其序列和结构与固氮酶相似, 反应时需要铁氧还蛋白(Ferredoxin, Fd)来催化还原Pchlide双键(Silva 2014)。尽管很早就确认藻类等植物中存在DPOR, 可以黑暗中合成叶绿素, 但对其特性并不了解。直到对红细菌属(*Rhodobacter*)突变体进行研究后才有了重要进展。红细菌是不产氧的光养生物, 一直作为研究细菌叶绿素生物合成的试验材料(Bauer等1993)。在球形红细菌(*R. sphaeroides*)和荚膜红细菌(*R. capsulatus*)突变体中确认了光合细菌非光依赖Pchlide还原过程必需的3个基因, 即*BchL*、*BchN*和*BchB* (Bollivar等1994; Burke等1993a; Coomber等1990; Yang和Bauer 1990; Zsebo和Hearst 1984)。之后, DPOR 3个亚基编码基因在产氧光合生物中确认, 即*ChlL*、*ChlN*和*ChlB* (Armstrong 1998; Fujita 1996; Nascimento等2016; Suzuki等1997)。其中, C*hlB*对植物叶绿素暗合成途径尤为重要(Fujita等2015)。

*ChlL*最初在地钱(*Marchantia polymorpha*)叶绿体基因组内被确认时命名为*frxC1* (Kohchi等

1988; Ohyama等1986), 因其开放阅读框(open reading frames, ORF)与固氮酶亚基编码基因*NifH*同源, 曾被认为参与固氮反应(Hearst等1985)。之后对突变体研究发现, 其参与黑暗中叶绿素合成, 与*BchL*相对应, 改为*ChlL* (Cheng等2005; Fujita等1992)。第2个亚基*ChlN*在鲍氏织线藻(*Plectonema boryanum*)中被克隆出, 最初称*gidA* (Adamson等1997; Suzuki和Bauer 1992), 后在莱茵衣藻突变体中被鉴定出功能(Choquet等1992; Fujita等1993)。第3个亚基*ChlB*是基于*BchB*被克隆并被定向诱变基础上, 证实其参与非光依赖型Pchlide还原(Fujita等1996; Li等1993; Liu等1993)。

之后, 绿藻(chlorophyta)、苔藓(bryophytes)、石松(lycophyta)以及一些裸子植物中均鉴定出*ChlLNB*同源物, 但在被子植物中找不到*ChlLNB*同源物(Breznenová等2010; Burke等1993b; Demko等2009; Fujita等1996; Richard等1994; Yamamoto等2011) 。

DPOR包含2个独立组分: L蛋白(ChlL或BchL)同源二聚体(α_2-homodimers)和NB蛋白(ChlNB或BchNB) $\alpha_2\beta_2$异源四聚体($\alpha_2\beta_2$-heterotetramer) (PDB数据库, 代码2YNM) (Bröcker等2008a, 2008b; Fujita和Yamakawa 2017; Nomata等2016)。L蛋白上有2个ATP结合位点和1个4Fe:4S氧化还原簇, NB蛋白上含有2个底物结合位点(Moser等2013; Nascimento等2016; Nomata等2008)。Karpinska等(1997)曾报道*ChlB*基因的RNA编辑在欧洲落叶松、欧日杂种落叶松(*L. eurolepis*)和樟子松(*P. sylvestris*)中具有物种及组织特异性, 这表明DPOR活性存在转录后调控。DPOR除了在黑暗中催化Pchlide还原外, 在光下也有助于叶绿素合成。其在一定程度上耐氧, 随着光强增加, 其催化效率逐渐降低, 当氧释放量达最大时, 催化效率最低(Fujita等1998; Shi和Shi 2006)。

在暗培养的被子植物中, 有光活性和非光活性2种类型的Pchlide (Schoefs 2000)。光活性Pchlide优先定位于PLBs (Böddi等1989; Selstam等1987), 通常与NADPH以及LPOR形成复合体, 见光后迅速光转化; 非光活性Pchlide优先定位于原类囊体(prothylakoids, PTs), 用于装载LPOR (Schoefs 2001)。在LPOR和DPOR同时存在的针叶植物中更复杂, DPOR复合体的准确定位、Pchlided分布是否受LPOR和DPOR调控以及这两种酶是否竞争Pchlide等问题都仍未解决(Demko等2009)。

2 黑暗条件下质体发育和光系统建成

黑暗条件下, 被子植物幼苗子叶黄化, 形成含有PLBs和PTs的白色体, 很少形成基粒(Mariani等1990)。而针叶树如云杉属*Picea* (Pavlovič等2016)、松属*Pinus* (Michel-Wolwertz和Bronchart 1974; Nikolić和Bogdanović 1972)、落叶松属*Larix* (Demko等2009; Mariani等1990)和杉木属*Cunninghamia* (Xue等2017)等植物在黑暗条件下可以合成叶绿素, 幼苗子叶外观呈绿色(图3-A和C)。质体超微结构也与被子植物不同, 通常形成含有PLBs的黄化叶绿体(etiochloroplast) (图3-B, 黑色无尾箭头; 图3-D, #指示PLBs), 黄化叶绿体中有分化明显的基粒结构(图3-B和D, 黑色带尾箭头所示)。

叶绿素对捕光蛋白复合体LHC (light-harvesting complex)和其他类囊体膜蛋白组装及稳定起重要作用(Kim等1994; Mariani等1990), 被子植物黑暗条件下不能合成叶绿素, 不能合成LHCII (Apel等1980; Bennett等1984)。见光后, 叶绿素开始合成, 才伴随LHC合成和光合器官组装(Klein和Mullet 1986)。而在叶绿素暗合成的植物类群中, 不仅叶绿素合成途径相关蛋白能正常合成表达, 光合作用相关蛋白及复合体也进行了合成及组装。对黑暗条件下发育的黑松幼苗子叶类囊体膜蛋白用SDS-PAGE电泳分析, 结果发现包括PSI、PSII、Cytb_6f和LCH在内的蛋白复合体都合成表达, 其表达量约为光照下表达量的1/4 (Shinohara等1992)。Western分析也显示, D1、PsbP、PsbS、LHCa和LHCb等蛋白在欧洲云杉和欧洲落叶松中均能正常合成(Maximová和Slováková 2014; Stolárik等2017)。用蓝绿温和胶电泳(blue-native PAGE)发现, 黑暗与光照培养条件并未造成杉木光反应蛋白复合体的显著区别; 进一步利用双向电泳技术, 发现光信号使更多LHCII蛋白出现在PSII复合体中, 而暗信号则使LHCII更倾向于在游离组分中出现(Xue等2017)。

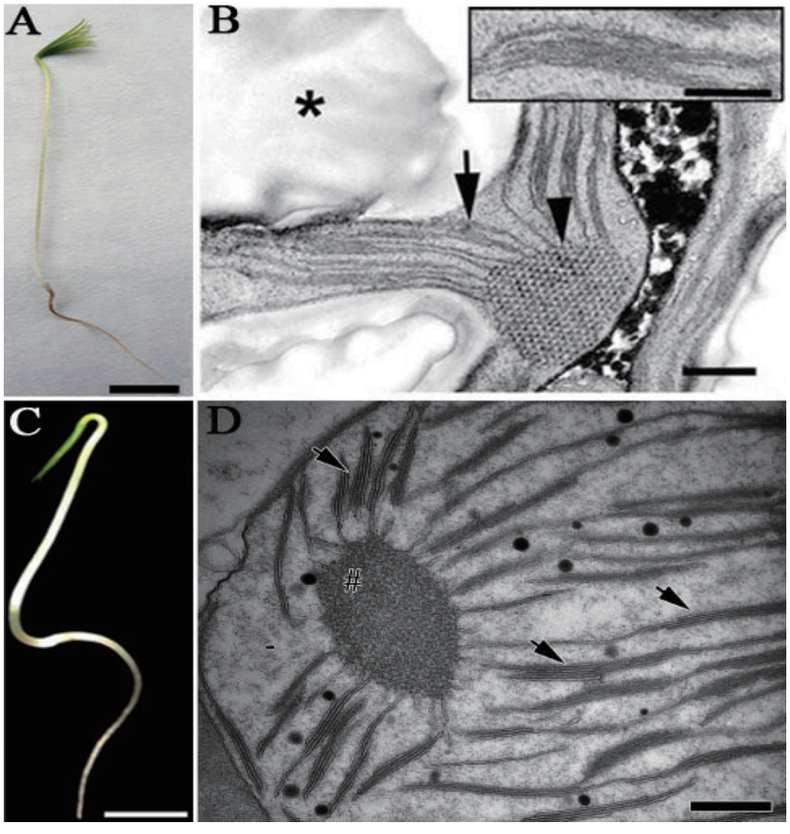

图3 裸子植物暗培养幼苗形态及质体超微结构

Fig.3 Morphology and ultrastructure of plastids in dark-grown gymnosperms seedlings

本图引自Demko等(2009)和Xue等(2017)文献并结合本课题组研究结果绘制。A: 暗培养14 d的欧洲云杉幼苗; 比例尺=1 cm; B: 暗培养欧洲云杉幼苗子叶质体超微结构; *: 淀粉粒; 黑色带尾箭头: 类囊体基粒片层; 黑色无尾箭头: PLBs; 放大框显示类囊体基粒片层结构; 比例尺=400 nm; C: 暗培养20 d的杉木(*Cunninghamia lanceolate*)幼苗, 比例尺=1 cm; D: 暗培养杉木幼苗子叶质体超微结构; 黑色箭头: 类囊体基粒片层; #: PLBs; 比例尺=500 nm。

黑暗下发育的针叶树幼苗光系统PSI活性正常, PSII放氧复合体(oxygen evolving complex, OEC)功能未活化, 见光后, O_2合成被激活(Muramatsu等2001; Oku和Tomita 1980; Pavlovič等2016; Shinohara等1992)。用低温发射光谱分析测定黑暗下发育的欧洲云杉子叶, 显示在685、695和735 nm有发射峰, 这三个峰值分别对应PSII内周天线蛋白CP43、CP47和PSI, 这表明色素分子能正常结合在两个光系统的蛋白复合体上(Stolárik等2017)。光系统活性测定也表明, 黑暗下建成的杉木子叶光系统PSI活性明显, 且PSI驱动的循环电子流(cyclic electron flow)传递速率显著高于PSII处电子传递速率(Xue等2017)。

3 植物进化过程中的叶绿素暗合成途径及其影响因素

3.1 叶绿素暗合成类群进化

内共生理论认为, 植物质体起源于蓝藻, 一些质体基因在进化过程中转变为核基因, 有些则丢失。LPOR在进化过程中被转移至核基因组, DPOR则保留在质体中, 有些类群则完全丢失DPOR (Burke等1993b; Gabruk等2012; Suzuki和Bauer 1995)。目前对进化过程中丢失DPOR的观点主要有以下几种: (1)氧气环境压力; (2)光照条件和温度的改变; (3)谱系特异性(lineage-specific)的基因丢失、RNA编辑以及非同义替换(nonsynonymous substitution)等

原因。另外, DPOR的ChlL亚基氨基酸序列及生化特性与LPOR有相似之处, 说明这两种酶在某些进化事件中曾出现汇合(Gabruk等2012; Gabruk和Mysliwa-Kurdziel 2015)。

DPOR编码基因最初起源于厌氧古生菌中的某个类固氮酶, 经过基因复制出现功能分化, 这也是DPOR对O_2敏感的原由。而LPOR最早出现在蓝藻中, 其产氧光合作用模式使得LPOR能在富氧条件下正常催化反应(Suzuki和Bauer 1995; Stolárik等2017)。除固氮酶外, DPOR还与叶绿素酸酯氧化还原酶(chlorophyllide oxidoreductase, COR)相似, COR在进化时间上早于DPOR, 在厌氧细菌叶绿素合成过程中催化C_7=C_8双键还原。进化过程中, COR从含氧光合生物中消失(Gupta和Khadka 2016; Vedalankar和Tripathy 2018), DPOR则在一些植物类群中保留了下来。植物从水生到陆生的进化中, 低等厌氧光合细菌通常只含有DPOR, 也有文献报道光合细菌中存在LPOR (Kaschner等2014), 藻类、地衣、苔藓、蕨类和裸子植物同时具有DPOR和LPOR, 被子植物则完全丢失DPOR, 只有LPOR (Fujita 1996; Masuda和Takamiya 2004; Xiong等1998)。但也有一些植物例外, 如藻类植物中华齿状藻(*Odontella sinensis*)、眼虫藻(*Euglena gracilis*)、蓝隐藻(*Guillardia theta*)和嗜热蓝藻(*Cyanidium caldarium*)丢失了DPOR。陆地植物中有些植物类群例如松叶蕨属(*Psilotum*)、买麻藤属(*Gnetumm*)、百岁兰属(*Welwitschia*)以及崖柏属(*Thuja*)植物由于缺失DPOR或出现了假基因化, 也不能合成DPOR (Hunsperger等2015; Kusumi等2006; Stolárik等2018; Ueda等2014) (图4)。

Lee等(2013)在本生烟(*Nicotiana benthamiana*)和拟南芥中发现伴侣蛋白(chaperone-like protein) CCP1对LPOR正常合成至关重要。在蓝藻中也发现有*CCP1*基因, 缺失*CPP1*基因的集胞藻(*Synechocystis* 6803)突变体*slr1918*出现高感光表型。与LPOR突变相比, DPOR突变体在大多数设置条件下均无表型, 但长时间低光或绿光条件会造成轻微的生长迟缓。例如, 地钱*ChlB*基因失活突变体在短日照条件下生长缓慢(Ueda等2014)。绿光条件下, *ChlLNB*基因在蓝藻*Fremyella diplosiphon*中表达量上调, 从LPOR的作用光谱来说, 绿光对光

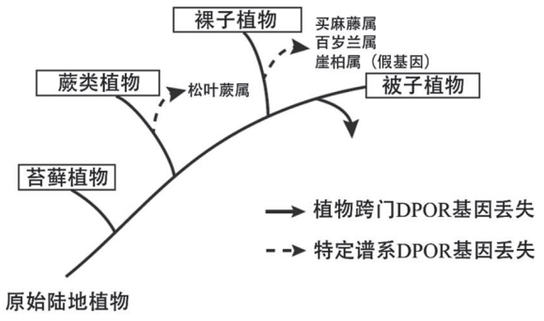

图4 DPOR基因在陆地植物中分布图
Fig.4 Schematic diagram illustrating the distribution of DPOR in land plants
根据Ueda等(2014)文献稍作修改。

反应是无效的, 在这种环境条件下, DPOR会补偿LPOR低活性状态(Shui等2009)。

在海洋光养生物领域, 中国科学院植物研究所解析了海洋浮游光合生物硅藻(Diatoms)的捕光天线蛋白岩藻黄素-叶绿素*a/c*蛋白复合体 (Fucoxanthin chlorophyll *a/c* protein, FCP)结构, 阐明了硅藻能在深水下进行高效能量传递的结构基础。硅藻FCP与绿藻以及高等植物的叶绿体*a/b*捕光天线蛋白序列同源性很低, FCP结合大量岩藻黄素和叶绿素*c*, 具有极强蓝绿光捕获能力和光保护能力来适应海洋环境(Wang等2019a)。

3.2 叶绿素暗合成影响因素

一些裸子植物虽然有DPOR编码基因, 但黑暗中发育的幼苗仍呈黄化状态。例如, 银杏(*Ginkgo biloba*)、日本落叶松(*L. kaempferi*)和樟子松等植物因缺乏有效的RNA编辑或其他原因导致无论光照和黑暗下均无法正常合成DPOR蛋白, 因此不能顺利进行叶绿素暗合成或暗合成能力极低(Armstrong 1998; Chinn和Silverthorne 1993; Drumm-Herrel和Mohr 1994; Karpinska等1997)。

光通常负调控DPOR各亚基, 例如莱茵衣藻ChlL亚基和光自养小球藻(*Chlorella protothecoides*) ChlB亚基在光下均有合成下降现象(Cahoon和Timko 2000; Shi和Shi 2006)。具活性的DPOR酶可能是针叶树幼苗忍受低光或黑暗环境的一个分子基础, 随着光强增加, 欧洲云杉DPOR各亚基丰度也降低(Stolárik等2018)。

周围环境例如低温也会影响叶绿素暗合成。例如, 低温下(8°C)日本黑松(*P. thunbergii*)子叶中叶绿素合成几乎完全被抑制(Muramatsu等2001); 欧洲云杉子叶在7°C下也不能合成叶绿素, 但DPOR编码基因在转录水平表达量并未受影响(Stolárik等2017)。银杏在黑暗中不能合成叶绿素, 其见光后的转绿过程同样受温度影响, 低温(10°C)下, 银杏子叶变绿过程明显慢于室温(20°C) (Skribanek等2010)。

DPOR对O_2敏感, 结合光照强度和O_2含量设置不同条件进行研究, 发现光照强度250~330 μmol·m^{-2}·s^{-1}范围内生长的蓝藻*Leptolyngbya boryana*突变体在O_2浓度高于3%的情况下DPOR不再起催化作用(Yamazaki等2006)。室温下增加黑暗中O_2含量, 欧洲云杉子叶中叶绿素合成量降低2/3 (Stolárik等2017)。

某些裸子植物叶绿素暗合成途径还与植物个体发育时期有关, 欧洲落叶松幼苗只在最初的发育阶段(7 d)合成叶绿素, 之后再延长黑暗时间(14 d), 叶绿素会迅速降解, 幼苗重新黄化; 见光后, GluTR和ALA合成又显著增强(Demko等2009)。因此, 欧洲落叶松在植物进化上可能介于裸子植物和被子植物中间的过渡地位(Demko等2009; Mariani等1990)。

另外, 裸子植物叶绿素合成的光、暗两条途径还具有明显的组织器官特异性, 不同的组织对光的响应模式不同。例如欧洲云杉非光依赖叶绿素只能在子叶中特异合成, 再培养的子叶愈伤组织中则检测不到Chl1B蛋白, 不能合成叶绿素(Balážová等2011), 后期次生针叶中也不再合成叶绿素(Stabel等1991; Stolárik等2017)。在杉木中也有类似现象, 杉木幼苗子叶可以通过非光依赖途径合成叶绿素(Xue等2017), 但2~3年生杉木顶生针叶黑暗中不再具有合成叶绿素的能力, 顶芽呈黄化状态(Wang等2019b)。

3.3 被子植物相关研究

被子植物在黑暗下不能合成叶绿素, 幼苗子叶通常黄化(Wang和Deng 2003)。拟南芥种子萌发过程中, 子叶中叶绿体发育过程可分为两个阶段: 黑暗中先形成白色体, 具典型PLBs结构; 见光后转变为叶绿体, PLBs转化为有效的类囊体膜, 同时启动叶绿素合成和光合复合体组装(Waters和Langdale 2009)。例如, 绿豆(*Vigna radiata*)在连续见光4 h后, 光合作用电子传递链基本形成, PSI和PSII出现明显活性(张汝民2005)。将被子植物例如拟南芥从光下转入黑暗后, 会导致叶绿素降解, 类囊体膜和叶绿体降解, 加快叶片衰老(Quirino等2000), 尤其当植株的一部分叶片被黑暗处理时, 衰老速度更快。目前已知PIFs蛋白直接或间接参与调控暗环境诱导的叶片衰老过程(Keech等2010; Liebsch和Keech 2016)。

被子植物莲(*Nelumbo nucifera*)胚芽在黑暗中可以变绿, 针对此现象, 有研究者对发育早期的莲蓬进行遮光处理后发现, 胚芽可继续发育, 但叶绿素合成受到严重抑制。同时, PCR扩增不到DPOR同源序列。所以, 莲胚芽的叶绿素合成只能通过光依赖途径进行(Ji等2001)。

4 结论与展望

低等植物类群例如藻类、地衣以及高等植物类群例如苔藓、蕨类和裸子植物叶绿素合成可以通过光、暗两条途径进行, 被子植物丢失了DPOR, 叶绿素合成只能通过光依赖途径进行。从进化的角度来看, 光依赖合成途径由于生物能量方式更保守且合成过程不需要额外消耗ATP, 因此更为进化。完全黑暗下, 能进行非光依赖叶绿素合成的植物类群与被子植物的质体结构及光系统发育过程有很大差异, 可能具有完全不同的暗-光形态建成转换机制, 这需要进一步研究证明。另外, 裸子植物非光依赖叶绿素合成及叶绿体发育模式具有明显的器官特异性, 次生针叶中叶绿素合成与幼苗子叶明显不同, 反而更接近被子植物, 这也需要后续研究。

参考文献(References)

Adamson H, Hiller R, Walmsley J (1997). Protochlorophyllide reduction and greening in angiosperms: an evolutionary perspective. J Photochem Photobiol B, 41 (3): 201–221

Aubert C, Vos MH, Mathis P, et al (2000). Intraprotein radical transfer during photoactivation of DNA photolyase. Nature, 405: 586–590

Apel K, Santel HJ, Redlinger TE, et al (1980). The protochlorophyllide holochrome of barley (*Hordeum vulgare* L.) isolation and characterization of the NADPH: protochlorophyllide oxidoreductase. Eur J Biochem, 111 (1): 251–258

Apitz J, Nishimura K, Schmied J, et al (2016). Posttranslational control of ALA synthesis includes GluTR degradation by Clp protease and stabilization by GluTR-binding protein. Plant Physiol, 170 (4): 2040–2051

Armstrong GA (1998). Greening in the dark: light-independent chlorophyll biosynthesis from anoxygenic photosynthetic bacteria to gymnosperms. J Photochem Photobiol B, 43 (2): 87–100

Awad MA (2008). Promotive effects of a 5-aminolevulinic acid-based fertilizer on growth of tissue culture-derived date palm plants (*Phoenix dactylifera* L.) during acclimatization. Sci Hortic (Amsterdam), 118 (1): 48–52

Balážová R, Blehová A, frickl V, et al (2011). Influence of irradiance on chlorophyll synthesis in *Picea abies* calli cultures. Biol Plant, 55 (1): 183–186

Bauer CE, Bollivar DW, Suzuki J (1993). Genetic analyses of photopigment biosynthesis in eubacteria: a guiding light for algae and plants. J Bacteriol, 175 (13): 3919–3925

Bennett J, Jenkins GI, Hartley MR (1984). Differential regulation of the accumulation of the light-harvesting chlorophyll *a/b* complex and ribulose bisphosphate carboxylase/oxygenase in greening pea leaves. J Cell Biochem, 25 (1): 1–13

Böddi B, Lindsten A, Ryberg M, et al (1989). On the aggregational states of protochlorophyllide and its protein complexes in wheat etioplasts. Physiol Plant, 76 (2): 135–143

Bollivar DW, Suzuki JY, Beatty JT, et al (1994). Directed mutational analysis of bacteriochlorophyll a biosynthesis in *Rhodobacter capsulatus*. J Mol Biol, 237 (5): 622–640

Breznenová K, Demko V, Pavlovič A, et al (2010). Light-independent accumulation of essential chlorophyll biosynthesis-and photosynthesis-related proteins in *Pinus mugo* and *Pinus sylvestris* seedlings. Photosynthetica, 48 (1): 16–22

Bröcker MJ, Virus S, Ganskow S, et al (2008a). ATP-driven reduction by dark-operative protochlorophyllide oxidoreductase from *Chlorobium tepidum* mechanistically resembles nitrogenase catalysis. J Biol Chem, 283 (16): 10559–10567

Bröcker MJ, Wätzlich D, Uliczka F, et al (2008b). Substrate recognition of nitrogenase-like dark operative protochlorophyllide oxidoreductase from *Prochlorococcus marinus*. J Biol Chem, 283 (44): 29873–29881

Burke DH, Alberti M, Hearst JE (1993a). *bchFNBH* bacteriochlorophyll synthesis genes of *Rhodobacter capsulatus* and identification of the third subunit of light-independent protochlorophyllide reductase in bacteria and plants. J Bacteriol, 175 (8): 2414–2422

Burke DH, Hearst JE, Sidow A (1993b). Early evolution of photosynthesis: clues from nitrogenase and chlorophyll iron proteins. Proc Natl Acad Sci USA, 90 (15): 7134–7138

Cahoon AB, Timko MP (2000). *yellow-in-the-dark* mutants of Chlamydomonas lack the CHLL subunit of light-independent protochlorophyllide reductase. Plant Cell, 12 (4): 559–568

Castelfranco PA, Rich PM, Beale SI (1974). The abolition of the lag phase in greening cucumber cotyledons by exogenous δ-aminolevulinic acid. Plant Physiol, 53 (4): 615–618

Cheng Q, Day A, Dowson-Day M, et al (2005). The *Klebsiella pneumoniae* nitrogenase Fe protein gene (*nifH*) functionally substitutes for the *chlL* gene in *Chlamydomonas reinhardtii*. Biochem Biophys Res Commun, 329 (3): 966–975

Chinn E, Silverthorne J (1993). Light-dependent chloroplast development and expression of a light-harvesting chlorophyll *a/b*-binding protein gene in the gymnosperm *Ginkgo biloba*. Plant Physiol, 103 (3): 727–732

Choquet Y, Rahire M, Girard-Bascou J, et al (1992). A chloroplast gene is required for the light‐independent accumulation of chlorophyll in *Chlamydomonas reinhardtii*. EMBO J, 11 (5): 1697–1704

Coomber S, Chaudhri M, Connor A, et al (1990). Localized transposon Tn5 mutagenesis of the photosynthetic gene cluster of *Rhodobacter sphaeroides*. Mol Microbiol, 4 (6): 977–989

Demko V, Pavlovič A, Valková D, et al (2009). A novel insight into the regulation of light-independent chlorophyll biosynthesis in *Larix decidua* and *Picea abies* seedlings. Planta, 230 (1): 165–176

Demko V, Pavlovič A, Hudak J (2010). Gabaculine alters plastid development and differentially affects abundance of plastid-encoded DPOR and nuclear-encoded GluTR and FLU-like proteins in spruce cotyledons. J Plant Physiol, 167 (9): 693–700

Denev ID, Yahubyan GT, Minkov IN, et al (2005). Organization of protochlorophyllide oxidoreductase in prolamellar bodies isolated from etiolated carotenoid-deficient wheat leaves as revealed by fluorescence probes. Biochim Biophys Acta, 1716 (2): 97–103

Drazic G, Bogdanovic M (2000). Gabaculine does not inhibit cytokinin-stimulated biosynthesis of chlorophyll in *Pinus nigra* seedlings in the dark. Plant Sci, 154 (1): 23–29

Dražić G, Mihailović N (1998). Chlorophyll accumulation in black pine seedlings treated with 5-aminolevulinic acid. Biol Plant, 41 (2): 277–280

Drumm-Herrel H, Mohr H (1994). Regulation by light of chlorophyll synthesis in the cotyledons of Scots pine (*Pinus sylvestris*) seedlings. Physiol Plant, 91 (2): 300–306

Forreiter C, van Cleve B, Schmidt A, et al (1991). Evidence for a general light-dependent negative control of NADPH-protochlorophyllide oxidoreductase in angiosperms.

Planta, 183 (1): 126–132
Frick G, Su Q, Apel K, et al (2003). An *Arabidopsis porB porC* double mutant lacking light-dependent NADPH: protochlorophyllide oxidoreductases B and C is highly chlorophyll-deficient and developmentally arrested. Plant J, 35 (2): 141–153
Fujita Y (1996). Protochlorophyllide reduction: a key step in the greening of plants. Plant Cell Physiol, 37 (4): 411–421
Fujita Y, Bauer CE (2003). The light-independent protochlorophyllide reductase: a nitrogenase-like enzyme catalyzing a key reaction for greening in the dark. In: Kadish KM, Smith KM, Guilard R (eds). The Porphyrin Handbook. Academic/Elsevier Science, London, 109–156
Fujita Y, Matsumoto H, Takahashi Y, et al (1993). Identification of a *nifDK*-like gene (ORF467) involved in the biosynthesis of chlorophyll in the cyanobacterium *Plectonema boryanum*. Plant Cell Physiol, 34 (2): 305–314
Fujita Y, Takagi H, Hase T (1996). Identification of the *chlB* gene and the gene product essential for the light-independent chlorophyll biosynthesis in the cyanobacterium *Plectonema boryanum*. Plant Cell Physiol, 37 (3): 313–323
Fujita Y, Takagi H, Hase T (1998). Cloning of the gene encoding a protochlorophyllide reductase: the physiological significance of the co-existence of light-dependent and-independent protochlorophyllide reduction systems in the cyanobacterium *Plectonema boryanum*. Plant Cell Physiol, 39 (2): 177–185
Fujita Y, Takahashi Y, Chuganji M, et al (1992). The *nifH*-like (*frxC*) gene is involved in the biosynthesis of chlorophyll in the filamentous cyanobacterium *Plectonema boryanum*. Plant Cell Physiol, 33 (1): 81–92
Fujita Y, Tsujimoto R, Aoki R (2015). Evolutionary aspects and regulation of tetrapyrrole biosynthesis in cyanobacteria under aerobic and anaerobic environments. Life, 5 (2): 1172–1203
Fujita Y, Yamakawa H (2017). Biochemistry of chlorophyll biosynthesis in photosynthetic prokaryotes. In: Hallenbeck PC (eds). Modern Topics in the Phototrophic Prokaryotes. Springer International Publishing AG, Cham, 67–122
Gabruk M, Grzyb J, Kruk J, et al (2012). Light-dependent and light-independent protochlorophyllide oxidoreductases share similar sequence motifs—in silico studies. Photosynthetica, 50 (4): 529–540
Gabruk M, Mysliwa-Kurdziel B (2015). Light-dependent protochlorophyllide oxidoreductase: phylogeny, regulation, and catalytic properties. Biochemistry, 54 (34): 5255–5262
Granick S (1970). Controls on chlorophylls synthesis in barley. Plant Physiol, 46 (2): 240–246
Gupta RS, Khadka B (2016). Evidence for the presence of key chlorophyll-biosynthesis-related proteins in the genus *Rubrobacter* (*Phylum Actinobacteria*) and its implications for the evolution and origin of photosynthesis. Photosynth Res, 127 (2): 201–218
Hanf R, Fey S, Schmitt M, et al (2012). Catalytic efficiency of a photoenzyme—an adaptation to natural light conditions. Chem Phys Chem, 13 (8): 2013–2015
Hearst JE, Alberti M, Doolittle RF (1985). A putative nitrogenase reductase gene found in the nucleotide sequences from the photosynthetic gene cluster of *R. capsulata*. Cell, 40 (1): 219–220
Hunsperger HM, Randhawa T, Cattolico RA (2015). Extensive horizontal gene transfer, duplication, and loss of chlorophyll synthesis genes in the algae. BMC Evol Biol, 15 (1): 16
Ji HW, Li LB, Kuang TY (2001). The chlorophyll biosynthesis in lotus embryo is light-dependent. Acta Bot Sin, 43 (7): 693–698
Karpinska B, Karpinski S, Hällgren J-E (1997). The *chlB* gene encoding a subunit of light-independent protochlorophyllide reductase is edited in chloroplasts of conifers. Curr Genet, 31 (4): 343–347
Kaschner M, Loeschcke A, Krause J, et al (2014). Discovery of the first light-dependent protochlorophyllide oxidoreductase in anoxygenic phototrophic bacteria. Mol Microbiol, 93 (5): 1066–1078
Kauss D, Bischof S, Steiner S, et al (2012). FLU, a negative feedback regulator of tetrapyrrole biosynthesis, is physically linked to the final steps of the Mg^{++}-branch of this pathway. FEBS Lett, 586 (3): 211–216
Keech O, Pesquet E, Gutierrez L, et al (2010). Leaf senescence is accompanied by an early disruption of the microtubule network in *Arabidopsis*. Plant Physiol, 154 (4): 1710–1720
Kim C, Apel K (2004). Substrate-dependent and organ-specific chloroplast protein import in planta. Plant Cell, 16 (1): 88–98
Kim J, Eichacker LA, Rudiger W, et al (1994). Chlorophyll regulates accumulation of the plastid-encoded chlorophyll proteins P700 and D1 by increasing apoprotein stability. Plant Physiol, 104 (3): 907–916
Klein RR, Mullet JE (1986). Regulation of chloroplast-encoded chlorophyll-binding protein translation during higher plant chloroplast biogenesis. J Biol Chem, 261 (24): 11138–11145
Kohchi T, Shirai H, Fukuzawa H, et al (1988). Structure and organization of *Marchantia olymorpha* chloroplast genome: IV. Inverted repeat and small single copy regions. J Mol Biol, 203 (2): 353–372
Kruse E, Mock HP, Grimm B (1995). Reduction of coproporphyrinogen oxidase level by antisense RNA synthesis leads to deregulated gene expression of plastid proteins and affects the oxidative defense system. EMBO J, 14 (15): 3712–3720
Kusumi J, Sato A, Tachida H (2006). Relaxation of functional constraint on light-independent protochlorophyllide oxidoreductase in *Thuja*. Mol Biol Evol, 23 (5): 941–948

Lee JY, Lee HS, Song JY, et al (2013). Cell growth defect actor1/chaperone-like protein of POR1 plays a role in stabilization of light-dependent protochlorophyllide oxidoreductase in *Nicotiana benthamiana* and *Arabidopsis*. Plant Cell, 25 (10): 3944–3960

Li J, Goldschmidt-Clermont M, Timko MP (1993). Chloroplast-encoded *chlB* is required for light-independent protochlorophyllide reductase activity in *Chlamydomonas reinhardtii*. Plant Cell, 5 (12): 1817–1829

Liebsch D, Keech O (2016). Dark-induced leaf senescence: new insights into a complex light-dependent regulatory pathway. New Phytol, 212 (3): 563–570

Liu XQ, Xu H, Huang C (1993). Chloroplast *chlB* gene is required for light-independent chlorophyll accumulation in *Chlamydomonas reinhardtii*. Plant Mol Biol, 23 (2): 297–308

Mariani P, De Carli ME, Rascio N, et al (1990). Synthesis of chlorophyll and photosynthetic competence in etiolated and greening seedlings of *Larix decidua* as compared with *Picea abies*. J Plant Physiol, 137 (1): 5–14

Masuda T, Fusada N, Oosawa N, et al (2003). Functional analysis of isoforms of NADPH: protochlorophyllide oxidoreductase (POR), PORB and PORC, in *Arabidopsis thaliana*. Plant Cell Physiol, 44 (10): 963–974

Masuda T, Takamiya KI (2004). Novel insights into the enzymology, regulation and physiological functions of light-dependent protochlorophyllide oxidoreductase in angiosperms. Photosynth Res, 81 (1): 1–29

Maximová N, Slováková Ľ (2014). Accumulation of photosynthetic pigments in *Larix decidua* Mill. and *Picea abies* (L.) Karst. cotyledons treated with 5-aminolevulinic acid under different irradiation. Photosynthetica, 52 (2): 203–210

McNellis TW, Deng XW (1995). Light control of seedling morphogenetic pattern. Plant Cell, 7 (11): 1749–1761

Memon SA, Hou X, Wang L, et al (2009). Promotive effect of 5-aminolevulinic acid on chlorophyll, antioxidative enzymes and photosynthesis of Pakchoi (*Brassica campestris* ssp. *chinensis* var. *communis* Tsen et Lee). Acta Physiol Plant, 31 (1): 51–57

Michel-Wolwertz M, Bronchart R (1974). Formation of prolamellar bodies without correlative accumulation of protochlorophyllide or chlorophyllide in pine cotyledons. Plant Sci Lett, 2 (1): 45–54

Mohanty S, Grimm B, Tripathy BC (2006). Light and dark modulation of chlorophyll biosynthetic genes in response to temperature. Planta, 224 (3): 692–699

Moser J, Lange C, Krausze J, et al (2013). Structure of ADP-aluminium fluoride-stabilized protochlorophyllide oxidoreductase complex. Proc Natl Acad Sci USA, 110 (6): 2094–2098

Muraki N, Nomata J, Ebata K, et al (2010). X-ray crystal structure of the light-independent protochlorophyllide reductase. Nature, 465 (7294): 110–114

Muramatsu S, Kojima K, Igasaki T, et al (2001). Inhibition of the light-independent synthesis of chlorophyll in pine cotyledons at low temperature. Plant Cell Physiol, 42 (8): 868–872

Nascimento S, Zou Y, Cheng Q (2016). Review of studies on the last enzymes in bacteriochlorophyll (*Bchl*) and chlorophyll (*Chl*) biosynthesis. Am J Plant Sci, 7 (12): 1639–1651

Nikolić D, Bogdanović M (1972). Plastid differentiation and chlorophyll synthesis in cotyledons of black pine seedlings grown in the dark. Protoplasma, 75 (3): 205–213

Nogaj LA, Beale SI (2005). Physical and kinetic interactions between glutamyl-tRNA reductase and glutamate-1-semialdehyde aminotransferase of *Chlamydomonas reinhardtii*. J Biol Chem, 280 (26): 24301–24307

Nogaj LA, Srivastava A, Van Lis R, et al (2005). Cellular levels of glutamyl-tRNA reductase and glutamate-1-semialdehyde aminotransferase do not control chlorophyll synthesis in *Chlamydomonas reinhardtii*. Plant Physiol, 139 (1): 389–396

Nomata J, Kondo T, Mizoguchi T, et al (2014). Dark-operative protochlorophyllide oxidoreductase generates substrate radicals by an iron-sulphur cluster in bacteriochlorophyll biosynthesis. Sci Rep, 4: 5455

Nomata J, Ogawa T, Kitashima M, et al (2008). NB-protein (BchN-BchB) of dark-operative protochlorophyllide reductase is the catalytic component containing oxygen-tolerant Fe-S clusters. FEBS Lett, 582 (9): 1346–1350

Nomata J, Terauchi K, Fujita Y (2016). Stoichiometry of ATP hydrolysis and chlorophyllide formation of dark-operative protochlorophyllide oxidoreductase from *Rhodobacter capsulatus*. Biochem Biophys Res Commun, 470: 704–709

Ohyama K, Fukuzawa H, Kohchi T, et al (1986). Chloroplast gene organization deduced from complete sequence of liverwort *Marchantia polymorpha* chloroplast DNA. Nature, 322 (6079): 572–574

Oku T, Tomita G (1980). Photoactivation of the latent O_2-evolving center in chloroplasts isolated from dark-grown spruce seedlings. Physiol Plant, 48 (1): 99–103

Oosawa N, Masuda T, Awai K, et al (2000). Identification and light-induced expression of a novel gene of NADPH-protochlorophyllide oxidoreductase isoform in *Arabidopsis thaliana*. FEBS Lett, 474 (2–3): 133–136

Pavlovič A, Demko V, Durchan M, et al (2009). Feeding with aminolevulinic acid increased chlorophyll content in Norway spruce (*Picea abies*) in the dark. Photosynthetica, 47 (4): 631–634

Pavlovič A, Stolárik T, Nosek L, et al (2016). Light-induced gradual activation of photosystem II in dark-grown Norway spruce seedlings. BBA-Bioenergetics, 1857 (6): 799–809

Plöscher M, Granvogl B, Reisinger V, et al (2009). Identifi-

cation of the N-termini of NADPH: protochlorophyllide oxidoreductase A and B from barley etioplasts (*Hordeum vulgare* L.). FEBS J, 276 (4): 1074–1081

Quirino BF, Noh YS, Himelblau E, et al (2000). Molecular aspects of leaf senescence. Trends Plant Sci, 5 (7): 278–282

Reinbothe C, Pollmann S, Phetsarath-Faure P, et al (2008). A pentapeptide motif related to a pigment binding site in the major light-harvesting protein of photosystem II, LHCII, governs substrate-dependent plastid import of NADPH: protochlorophyllide oxidoreductase A. Plant Physiol, 148 (2): 694–703

Reinbothe C, El Bakkouri M, Buhr F, et al (2010). Chlorophyll biosynthesis: spotlight on protochlorophyllide reduction. Trends Plant Sci, 15 (11): 614–624

Richard M, Tremblay C, Bellemare G (1994). Chloroplastic genomes of *Ginkgo biloba* and *Chlamydomonas moewusii* contain a *chlB* gene encoding one subunit of a light-independent protochlorophyllide reductase. Curr Genet, 26 (2): 159–165

Sarma R, Barney BM, Hamilton TL, et al (2008). Crystal structure of the L protein of Rhodobacter sphaeroides light-independent protochlorophyllide reductase with MgADP bound: a homologue of the nitrogenase Fe protein. Biochemistry, 47 (49): 13004–13015

Schoefs B (2000). The light-dependent and light-independent reduction of protochlorophyllide a to chlorophyllide *a*. Photosynthetica, 36 (4): 481–496

Schoefs B (2001). The protochlorophyllide–chlorophyllide cycle. Photosynth Res, 70 (3): 257–271

Schoefs B, Franck F (2003). Protochlorophyllide reduction: mechanisms and evolution. Photochem Photobiol, 78 (6): 543–557

Selstam E, Widell A, Johansson LB-Å (1987). A comparison of prolamellar bodies from wheat, Scots pine and Jeffrey pine. Pigment spectra and properties of protochlorophyllide oxidoreductase. Physiol Plant, 70 (2): 209–214

Shi C, Shi X (2006). Expression switching of three genes encoding light-independent protochlorophyllide oxidoreductase in *Chlorella protothecoides*. Biotechnol Lett, 28 (4): 261–265

Shinohara K, Murakami A, Fujita Y (1992). Biochemical characteristics of thylakoid membranes in chloroplasts of dark-grown pine cotyledons. Plant Physiol, 98 (1): 39–43

Shui J, Saunders E, Needleman R, et al (2009). Light-dependent and light-independent protochlorophyllide oxidoreductases in the chromatically adapting cyanobacterium *Fremyella diplosiphon* UTEX 481. Plant Cell Physiol, 50: 1507–1521

Silva PJ (2014). With or without light: comparing the reaction mechanism of dark-operative protochlorophyllide oxidoreductase with the energetic requirements of the light-dependent protochlorophyllide oxidoreductase. PeerJ, 2: e551

Skinner JS, Timko MP (1999). Differential expression of genes encoding the light-dependent and light-independent enzymes for protochlorophyllide reduction during development in loblolly pine. Plant Mol Biol, 39 (3): 577–592

Skribanek A, Solymosi K, Hideg É, et al (2010). Light and temperature regulation of greening in dark-grown ginkgo (*Ginkgo biloba*). Physiol Plantarum, 134 (4): 649–659

Smith JH, Kupke DW (1956). Some properties of extracted protochlorophyll holochrome. Nature, 178 (4536): 751–752

Solymosi K, Smeller L, Ryberg M, et al (2007). Molecular rearrangement in POR macrodomains as a reason for the blue shift of chlorophyllide fluorescence observed after phototransformation. Biochim Biophys Acta, 1768 (6): 1650–1658

Solymosi K, Schoefs B (2010). Etioplast and etio-chloroplast formation under natural conditions: the dark side of chlorophyll biosynthesis in angiosperms. Photosynth Res, 105 (2): 143–166

Sorigué D (2017). An algal photoenzyme converts fatty acids to hydrocarbons. Science, 357: 903–907

Stabel P, Sundås A, Engström P (1991). Cytokinin treatment of embryos inhibits the synthesis of chloroplast proteins in Norway spruce. Planta, 183 (4): 520–527

Stolárik T, Hedtke B, Šantrůček J, et al (2017). Transcriptional and post-translational control of chlorophyll biosynthesis by dark-operative protochlorophyllide oxidoreductase in Norway spruce. Photosynth Res, 132 (2): 165–179

Stolárik T, Nožková V, Nosek L, et al (2018). Dark chlorophyll synthesis may provide a potential for shade tolerance as shown by a comparative study with seedlings of European larch (*Larix decidua*) and Norway spruce (*Picea abies*). Trees, 32 (4): 951–965

Su Q, Frick G, Armstrong G, et al (2001). POR C of *Arabidopsis thaliana*: a third light-and NADPH-dependent protochlorophyllide oxidoreductase that is differentially regulated by light. Plant Mol Biol, 47 (6): 805–813

Suzuki JY, Bauer CE (1992). Light-independent chlorophyll biosynthesis: involvement of the chloroplast gene *chlL* (*frxC*). Plant Cell, 4 (8): 929–940

Suzuki JY, Bauer CE (1995). A prokaryotic origin for light-dependent chlorophyll biosynthesis of plants. Proc Natl Acad Sci USA, 92 (9): 3749–3753

Suzuki JY, Bollivar DW, Bauer CE (1997). Genetic analysis of chlorophyll biosynthesis. Annu Rev Genet, 31 (1): 61–89

Ueda M, Tanaka A, Sugimoto K, et al (2014). *chlB* requirement for chlorophyll biosynthesis under short photoperiod in *Marchantia polymorpha* L. Genome Biol Evol, 6 (3): 620-628

Vedalankar P, Tripathy BC (2018). Evolution of light-independent protochlorophyllide oxidoreductase. Protoplasma, 256 (2): 293–312

Wang H, Deng XW (2003). Dissecting the phytochrome A-de-

pendent signaling network in higher plants. Trends Plant Sci, 8 (4): 172–178

Wang Q, Liu S, Li X, et al (2019b). Expression of genes in new sprouts of *Cunninghamia lanceolata* grown under dark and light conditions. J Plant Growth Regul, 39 (1): 481–491

Wang W, Yu LJ, Xu C, et al (2019a). Structural basis for blue-green light harvesting and energy dissipation in diatoms. Science, 363 (6427): eaav0365

Waters MT, Langdale JA (2009). The making of a chloroplast. EMBO J, 28 (19): 2861–2873

Xiong J, Inoue K, Bauer CE (1998). Tracking molecular evolution of photosynthesis by characterization of a major photosynthesis gene cluster from *Heliobacillus mobilis*. Proc Natl Acad Sci USA, 95 (25): 14851–14856

Xue X, Wang Q, Qu Y, et al (2017). Development of the photosynthetic apparatus of *Cunninghamia lanceolata* in light and darkness. New Phytol, 213 (1): 300–313

Yamamoto H, Kurumiya S, Ohashi R, et al (2011). Functional evaluation of a nitrogenase-like protochlorophyllide reductase encoded by the chloroplast DNA of *Physcomitrella patens* in the cyanobacterium *Leptolyngbya boryana*. Plant Cell Physiol, 52 (11): 1983–1993

Yamamoto H, Kusumi J, Yamakawa H, et al (2017). The effect of two amino acid residue substitutions via RNA editing on dark-operative protochlorophyllide oxidoreductase in the black pine chloroplasts. Sci Rep, 7 (1): 2377

Yamazaki S, Nomata J, Fujita Y (2006). Differential operation of dual protochlorophyllide reductases for chlorophyll biosynthesis in response to environmental oxygen levels in the cyanobacterium *Leptolyngbya boryana*. Plant Physiol, 142 (3): 911–922

Yang J, Cheng Q (2004). Origin and evolution of the light-dependent protochlorophyllide oxidoreductase (LPOR) genes. Plant Biol, 6 (5): 537–544

Yang Z, Bauer C (1990). *Rhodobacter capsulatus* genes involved in early steps of the bacteriochlorophyll biosynthetic pathway. J Bacteriol, 172 (9): 5001–5010

Zhang R (2005). Study on physiological and biochemical mechanism of plastids during early stage of seedling de-etiolation of *Phaseolus radiatus* L. (dissertation). Beijing: Beijing Forsetry University (in Chinese with English abstract) [张汝民(2005). 绿豆幼苗脱黄化初期质体发育生理生化机制的研究(学位论文). 北京: 北京林业大学]

Zhang S, Heyes DJ, Feng L, et al (2019). Structural basis for enzymatic photocatalysis in chlorophyll biosynthesis. Nature, 574 (7780): 722–725

Zhong S, Zhao M, Shi T, et al (2009). EIN3/EIL1 cooperate with PIF1 to prevent photo-oxidation and to promote greening of *Arabidopsis* seedlings. Proc Natl Acad Sci USA, 106 (50): 21431–21436

Zsebo KM, Hearst JE (1984). Genetic-physical mapping of a photosynthetic gene cluster from *R. capsulata*. Cell, 37 (3): 937–947

Progress on light-independent chlorophyll biosynthesis in plants

XUE Xian[1,*], WAN Yinglang[2], SHI Xinlong[1], ZENG Fanzi[1]

[1]*College of Agriculture, Henan University of Science and Technology, Luoyang, Henan 471000, China*
[2]*Institute of Tropical Agriculture and Forestry, Hainan University, Haikou 570228, China*

Abstract: The light-independent protochlorophyllide reductase (DPOR) was related to the ability of plants to synthesize chlorophyll in complete darkness. As an ancient enzyme, DPOR has been reserved in photosynthetic organisms from bacteria to gymnosperm, while missed in angiosperm. Therefore, angiosperm could not synthesize chlorophyll in darkness. In this review, we summarize recent advances concerning chlorophyll biosynthesis pathway. We also discuss the dark chlorophyll synthesis in the aspect of evolution and influencing factors, providing a collection of references for further researches in plants skotomorphogenesis and photosynthetic apparatus development during dark-light transition process.

Key words: dark synthesis of chlorophyll; light-independent protochlorophyllide reductace (DPOR); photosystem development

Received 2019-09-25 Accepted 2020-06-19

This work was supported by the Doctor Fund of Henan University of Science and Technology (13480079) and Experimental Technology Development Fund of Henan University of Science and Technology (1819019).

*Corresponding author (xuexian2011@126.com).