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叶绿素合成调控研究进展

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摘要: 万物生长靠太阳, 植物、海洋藻类、光合细菌等利用光合作用将光能转变为化学能为地球生物提供能量。叶绿素、类胡萝卜素等光合色素在捕光天线复合体中吸收光能以驱动电子传递, 是光合作用中不可或缺的组分。因此, 研究叶绿素生物合成调控机制是提高光合作用效率、解决粮食危机、能源危机以及改善地球生态环境的重要科技着力点。叶绿素的重要性及其光敏感性前体物质潜在的氧化胁迫危害, 使得叶绿素合成过程在多个层面被精细调控。在叶绿素合成与叶绿体发育中, 光作为最重要的环境信号发挥着决定性作用, 激素、叶绿体反馈信号等内源信号亦不可或缺。经过近几十年的探究, 前人对叶绿素合成调控机制有了更为全面的认识, 包括: (1) 叶绿素代谢酶活性的调节; (2) 叶绿体反馈信号的作用; (3) 细胞核内的转录调控。这些研究分别在叶绿体内部、叶绿体与细胞核之间、细胞核内部三个不同的空间层面揭示了叶绿素合成的调控机制。叶绿素生物合成是一个令人兴奋的领域, 对植物生物学、农业、合成生物学甚至现代医学都具有重要意义。通过采用综合的多组学方法以及尖端技术和跨学科方法, 可以更加全面地了解叶绿素生物合成。这种更深入的了解将使我们能够在未来更有效地利用其潜力。

关键词: 光合作用; 叶绿素合成; 光信号; 反馈信号

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Advances in the regulation mechanism of chlorophyll synthesis

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Abstract: Plants, algae, and photosynthetic bacteria convert light into chemical energy through photosynthesis, providing energy for life on Earth. Photosynthetic pigments such as chlorophyll and carotenoids absorb light energy

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to drive electron transport, and are essential for photosynthesis. Therefore, studying the regulatory mechanism of chlorophyll biosynthesis is crucial to promoting photosynthesis efficiency, addressing the food and energy crises, and improving the Earth's ecological environment. The importance of chlorophyll and the potential oxidative stress of photosensitive precursors necessitate precise regulation of chlorophyll synthesis at multiple levels. Light plays a crucial role as the primary environmental signal, while internal signals such as hormones and chloroplast retrograde signals are also essential in chlorophyll synthesis and chloroplast development. After decades of research, we now have a more comprehensive understanding of the regulatory mechanism of chlorophyll synthesis, including the regulation of chlorophyll metabolic enzyme activity, the role of chloroplast retrograde signals, and the transcriptional regulation in the nucleus. These studies have revealed the regulatory mechanism of chlorophyll synthesis at three distinct spatial levels: within chloroplasts, between chloroplasts and nuclei, and within nuclei. Chlorophyll biosynthesis is an exciting field that holds significant implications for plant biology, agriculture, synthetic biology, and even modern medicine. By employing comprehensive multi-omics approaches alongside cutting-edge technologies and interdisciplinary methods, we can gain a more holistic understanding of chlorophyll biosynthesis. This deeper insight will enable us to harness its potential.

Key words: photosynthesis; chlorophyll synthesis; light signal; retrograde signal

1 叶绿素合成通路

叶绿素广泛存在于植物、藻类以及光合细菌中,在少量原生动物,比如绿眼虫中也发现了叶绿素的存在,其在光合作用的光能捕获以及能量传递过程中发挥着重要作用。光合细菌中的类似色素被称为细菌叶绿素,其结构有别于植物和藻类中的叶绿素,但同样具有捕获光能的作用^[1-2]。叶绿素不仅在自然界光合作用中十分重要,同时作为天然色素原料以及天然医用原料,在食品加工、抗氧化、抗炎症、抗癌等医疗保健领域的功能与应用也正在被逐渐关注和重视^[3-5]。目前植物和藻类中发现的叶绿素总共存在5种类型,分别是叶绿素a、叶绿素b、叶绿素c(包含c1、c2和c3)、叶绿素d以及叶绿素f^[6-8]。叶绿素、血红素(Heme)、西罗血红素以及光敏胆色素均来自于四吡咯代谢通路。叶绿素合成是一个十分复杂的代谢通路,包括5-氨基乙酰丙酸(5-aminolevulinic acid, 5-ALA)的合成、四吡咯共同的代谢部分、叶绿素合成分支以及叶绿素循环四个部分,相关基因及其编码酶均已被鉴定^[9-13]。叶绿素合成通路中的代谢酶多达十几种,深入探究这些催化酶的生化活性以及调控机制是一直以来的热点。依据前人的研究,目前认为叶绿素合成过程中存在三个关键调控节点:(1)5-ALA合成是整个四吡咯代谢通路的限速步骤;(2)叶绿素与血红素分流节点是螯合镁离子开启叶绿素分支的第一步反应;(3)严格依赖光照激活的原叶绿素酸酯氧化酶(light-dependent pchlide oxidoreductase, LPOR)是被子植物叶绿素合成的必需步骤^[14]。

近些年,国内有关叶绿素合成代谢酶的研究取得了诸多进展。(1)谷氨酰-tRNA还原酶(Glu-tRNA reductase, GluTR)是催化谷氨酰-tRNA还原成5-ALA的关键步骤,也是叶绿素合成的限速步骤。国内研究组解析了拟南芥GluTR与其结合蛋白GBP(GluTR binding protein)、Flu(fluorescent in blue light)的复合物晶体结构,揭示了GBP以及Flu对GluTR活性的调控作用^[15-16]。(2)光依赖型的原叶绿素酸酯氧化还原酶LPOR将黑暗中积累的原叶绿素酸酯(protochlorophyllide, Pchlde)在光照条件下转变为叶绿素酸酯(chlorophyllide, Chlide),是光暗转换过程中十分关键的催化酶。国内外研究组分别解析了蓝藻LPOR酶单体以及LPOR-NADPH蛋白复合体的晶体结构,揭示了POR驱动催化反应的作用机理^[17-18]。(3)GUN4(genome uncoupled 4)蛋白是镁螯合酶(magnesium chelatase, MgCh)调控亚基,通过结合底物原卟啉IX(protochlorophyllide, Proto IX)与产物镁原卟啉IX(Mg-protoporphyrin IX, Mg-Proto IX)调控MgCh活性,催化叶绿素合成分支的第一步反应。我国科研人员发现,莱茵衣藻CrGUN4可结合Heme分支代谢产物胆色素(bilins),促进MgCh活性及维持MgCh催化亚基CHLH1的稳定性,调控叶绿素合成^[19-20]。不同于陆地植物,海洋藻类的光合作用依赖能吸收蓝绿光波段的叶绿素c、岩藻黄素等,但是催化叶绿素c合成的酶并未被解析。最新突破性研究进展是在硅藻中揭示了叶绿素c合成酶编码基因CHLC(Chl c synthase),并解析了CHLC的催化机制^[21]。

2 叶绿素合成调控研究进展

2.1 四吡咯代谢“内调控”(叶绿体内调控)

前人在叶绿素代谢酶活性的调控方面取得了诸多进展, 尤其关注关键代谢酶 GluTR、镁螯合酶 MgCh 调控亚基 GUN4 以及 POR^[14,22]。近期研究发现, MORF2 (multiple organellar RNA editing factor 2) 和 MORF9 与 GUN4 互作促进 GUN4 复合体积聚, 以不依赖于 GUN4 的方式调控 MgCh 活性^[23]。同时, 拟南芥 MORF2 和 MORF9 也被证实具有 Holdase 活性, 在体外有效减缓 PORB 聚集, 维持 POR 稳定^[23]。此外, PCD8 (programmed cell death 8) 与四吡咯代谢酶 HEMC、CHLD 以及 PORC 互作, 也与分子伴侣 ClpC1 互作, 调控叶绿素合成^[24]。值得一提的是, 德国 Bernhard Grimm 课题组近些年取得了一系列研究进展, 发现: cpSRP43 (chloroplast signal recognition particle 43) 和 cpSRP54 对 GluTR、GUN4 蛋白质稳定性起到保护作用^[25-26]; 定位于类囊体膜的 BCMs (balance of chlorophyll metabolism) 与 GUN4 互作调控镁螯合酶活性促进叶绿素合成, 维持叶绿素合成与降解的平衡^[27]; 血红素加氧酶 FC2 被证实通过与 FLU-POR-GluTR 复合体中的 POR 互作抑制 GluTR 活性^[28]。

2.2 叶绿体反馈信号调控(叶绿体与细胞核之间的互作调控)

内共生而来的叶绿体, 其发育及基因表达均受到细胞核控制, 研究人员将细胞核向叶绿体传递的信号称为正向信号 (anterograde signaling)。与此同时, 叶绿体也会释放信号调控细胞核相关基因表达, 该逆行信号被称为反馈信号 (retrograde signaling)^[29-32]。Chory 实验室通过正向遗传筛选克隆到反馈信号因子 GUN1~GUN6 (genome uncoupled 1~6), 使得叶绿体反馈信号调控植物生长发育的作用机制成为研究热点^[33-34]。叶绿体既保留了自身编码蛋白的功能, 同时接收细胞核编码蛋白。前人将叶绿体编码光合相关蛋白的基因称为 *PhAPGs* (photosynthesis-associated plastid genes), 将细胞核编码光合相关蛋白的基因称为 *PhANGs* (photosynthesis-associated nuclear genes); 其中, 叶绿素合成相关基因 (比如 *HEMA1/FLU/PPO1/GUN5/PORA/PORB/PORC* 等) 均属于 *PhANGs* 基因^[35]。越来越多的研究表明, 叶绿体反馈信号在叶绿素合成过程中至关重要。在莱茵衣藻中发现, Heme 衍生代谢物——胆色素对光依赖性的转绿以及光合作用至关重要^[36-38]。此外, Heme 作为很多

蛋白的结合色素不仅仅分布在叶绿体中, 也能够迁移至线粒体、细胞质甚至细胞核中行使功能^[14]。尽管在脊椎动物、酵母和蠕虫中已经发现了几种 Heme 转运因子, 但迄今为止在植物中还没有相关报道, 因此 Heme 传递反馈信号的作用机制并不清楚。GUN1 整合叶绿体中的多个反馈信号, 调控细胞核 *PhANGs* 基因表达^[39-40]。研究发现, GUN1 不仅在叶绿体内部调控叶绿体发育, 同时调控叶绿体前体蛋白转运, 影响 ClpD 及叶绿素合成相关蛋白 GluTR、GBP 等向质体转运^[41]。但是, 目前对于 GUN1 蛋白的生物学性质并没有明确定义, 因而其介导的反馈信号如何传导也尚未可知。此外, 最新研究显示, 不同类型的反馈信号交叉调控叶绿体发育, 水稻中的四吡咯核心因子 OsGUN4/OsGUN5 可调控 *PhAPGs* 表达, 参与叶绿体反馈信号传递^[42]。近些年, ROS 介导的反馈信号在叶绿体发育与叶绿素合成中的作用逐渐引起关注。叶绿体中产生的 ROS 具有两面性: 一方面, ROS (比如单线态氧 (1O_2)) 作为毒性物质, 与植物生物大分子反应破坏其功能, 尤其是在高光以及胁迫等条件下; 另一方面, ROS 作为重要的信号分子, 调控植物的生长发育, 如种子休眠、叶片转绿等过程^[43-47]。高光照处理材料转录组数据表明, 叶绿体 1O_2 是拟南芥响应高光最主要的 ROS 种类^[48]。同时, 国外实验室研究发现, EX1/EX2 (Executer1/Executer2) 介导的叶绿体反馈信号调控幼苗早期叶绿素合成^[49]。*flu* 突变体和 *pif1 pif3* 突变体在黑暗中积累了大量光敏感物质 Pchl_{ide}, 见光后迅速产生大量 1O_2 , 此时 EX1/EX2 介导的 1O_2 信号导致细胞死亡、幼苗黄化甚至白化^[50-52]。最新研究发现, “ 1O_2 受体” EX1 蛋白响应 1O_2 信号由叶绿体转移至细胞核, 与转录因子 WRKYs 相互作用调控幼苗转绿^[53]。

2.3 光信号与激素信号的转录调控作用(细胞核内调控)

光的能量属性与环境信号属性使其在植物叶绿体发育中发挥着不可或缺的作用。与此同时, 光信号与多种激素信号共同调控叶绿素合成, 形成了十分复杂的调控网络。光信号与激素信号的调控作用主要体现在转录水平, 但随着生物学技术的不断进步, 在染色质水平以及其他方面也取得了一些研究进展, 比如最新研究发现蓝光受体 CRY2 介导的 mRNA-m⁶A 修饰调控叶绿素稳态^[54-55]。光信号转导核心因子 PIFs (photochrome interacting factors) 与 HY5 (elongated hypocotyl 5)、油菜素内酯 (brassinolide,

BR) 信号中的 BZR1 (brassinazole-resistant 1) 与 BES1 (Bri1-EMS-suppressor 1) 以及乙烯信号中的 EIN3 (ethylene insensitive 3) 等转录因子均调控叶绿素合成基因表达, 而另一类转录因子 GLK1/GLK2 (golden-like 1/2)、GATA 以及 CGA1/GNL (cytokinin-induced GATA1/GNC-like) 则实现了对光信号以及激素信号等多种内外信号的整合, 尤其是 GLK1/GLK2, 对叶绿素合成以及叶绿体发育至关重要^[56-58]。

光信号对叶绿素合成的调控体现在对其黑暗下的抑制和光照下的激活。一方面是黑暗条件下, 光信号缺失促使光信号负向调控因子 PIFs 和 COP1 (constitutive photomorphogenic 1) 等大量积累, 抑制叶绿素合成与叶绿体发育。另一方面是光照条件下, PIFs 被磷酸化降解, COP1 活性被光受体抑制, 而光信号正向调控因子 HY5、FHY3/FAR1 (far-red elongated hypocotyl 3/far-red-impaired response 1) 等大量积累, 激活四吡咯代谢基因表达, 促进叶绿素合成^[59-61]。在黑暗条件下, *pifq* 突变体 (缺失 *PIF1*、*PIF3*、*PIF4* 和 *PIF5*) 中大部分四吡咯代谢基因转录水平显著上升, 表明 PIFs 在转录水平调控叶绿素合成中具有重要作用^[62-63]。首先, PIFs 通过直接或间接的作用方式抑制 *HEMA1*、*CHLH* 和 *GUN4* 等基因表达, 促进 *PORA* 表达^[53,64]。其次, 在黑暗条件下, PIFs 协同 COP1 促进 HY5 降解, 进而削弱 HY5 对 *TPB* 基因的转录激活作用^[65], 且 PIFs 调控 *CHLH*、*CAB* 等基因的分布位置重置 (即由细胞核内部转移至细胞核膜外缘的现象), 以及招募染色质重塑因子 BRM (Brahma)、去乙酰化酶 HDA15/HDA19 等在染色质水平调控叶绿素合成^[66-69]。此外, 在黑暗中, 转录因子 RVE1 (Reveille1) 直接结合 *PORA* 启动子促进其表达, 同时抑制 *GSA2*、*CHLH* 以及 *CRD1* 表达, 进而抑制叶绿素合成^[70]。在光照条件下, 转录因子 HY5 对叶绿素的合成至关重要, 其功能缺失会导致叶绿素含量降低。HY5 被证实可直接结合 *CHLH* 以及 *PORC* 等基因启动子区的 G-box 结构域从而激活其表达, 并通过间接方式促进 *HEMA1* 表达, 进而促进叶绿素合成^[64]。此外, 转录因子 FHY3/FAR1 通过直接结合 *HEMB1* 启动子, 以及间接调控 *GUN4* 和 *CHLH* 等基因表达, 促进叶绿素合成^[71-72]。光信号转导核心因子 PIFs 与 HY5 的这种相互作用, 拮抗调控四吡咯代谢基因转录水平, 使幼苗在黑暗中不会过度积累光敏感物质 (比如 Pchl_{ide}), 同时保证了见光后 POR 酶被迅速激活, 进而避免光漂白。

激素信号对叶绿素合成的调控作用主要体现在对叶绿体发育核心转录因子 GLK1/GLK2、GATA 以及 CGA1/GNL 等的调控作用以及其与光信号之间的交叉互作。GLK1/GLK2、GNC、GNL/CGA1 以及 GATA15、GATA16、GATA17 和 GATA17L 等被报道直接调控 *GUN4*、*CHLH* 和 *CHLM* 等基因表达, 正向调控叶绿素合成, 这些转录因子对叶绿体发育以及叶绿素合成的调控作用具有冗余性^[56,61]。黑暗下 BR 抑制叶绿素合成, 一方面是其转录因子 BZR1 以及 BZR2/BES1 直接抑制 *GATA2*、*GATA4* 以及 *GLK1/2* 转录^[73-74]; 另一方面, BZR1/BES1 与 PIF4 互作, 抑制 *GLK1/GLK2* 以及叶绿素合成基因表达^[75]。此外, 黑暗下 COP1 促进磷酸化形式的 BZR1 (非活性形式) 降解, 促使有活性的非磷酸化 BZR1 在黑暗中发挥作用^[76]。近年国内研究发现, 光信号因子 HY5 与 BZR1 互作并抑制其功能^[77]。与此同时, 光下 HY5 促进 BR 负向因子 BIN2 激酶稳定性, 削弱 BR 对叶绿体发育的抑制作用^[78]。最新研究表明, 光下 BIN2 通过促进 GLK1 磷酸化激活叶绿体发育^[79], 并且 BPG4 (Brz-insensitive-pale green 4 (*bpg4*)) 响应 BR 和光信号, 通过与 GLK1 互作抑制其转录活性^[80]。在黑暗条件下, GA 信号负向因子 DELLA 蛋白, 一方面直接促进 *POR* 基因表达以及蛋白积累, 使植物保持较高的 POR 酶活性^[81]; 另一方面与 PIFs 互作并促进其降解, 解除其对 *GNC/GNL* 的转录抑制^[82]。黑暗中乙烯信号转录因子 EIN3/EIL1 直接结合启动子促进 *PORA* 和 *PORB* 表达, 与 PIFs 协同抑制叶绿体发育。同时, EIN3 蛋白被光信号调控: COP1 在黑暗条件下降解 EBF1/2 (EIN3-binding F-box 1/2) 促进 EIN3 蛋白稳定, PHYB 则在光下促进 EBF1/2 与 EIN3 互作促进其降解^[83-86]。此外, Auxin 信号转导通路中的转录因子 ARF (auxin response factor) 以及 CK 信号转导通路中的 B-type ARRs (*Arabidopsis* response regulators) 等转录因子直接结合 *GLK1*、*CAB* 等基因启动子, 调控这些基因表达, 促进叶绿素合成^[87-89]。叶绿素合成是光合作用的重要环节, 但过多叶绿素的积累对于果实的成熟与着色是十分不利的, 因此叶绿素的降解对于叶绿素含量的调控至关重要。我国是园艺大国, 近些年随着生物技术的进步, 在草莓、香蕉、番茄、荔枝、柑橘等果实成熟过程中叶绿素降解调控方面取得了诸多研究进展, 发现了一些重要的转录调控因子, 比如 CsMADS3、MaMYB60 等, 为进一步提高果实品质奠定了重要的理论基础^[90-94]。

3 开放性问题与展望

随着人们对叶绿素合成调控机制的深入探究, 很多新的调控机制被逐渐发现。

研究发现, 除了四吡咯代谢通路中间产物, 叶绿体中的其他代谢通路中间产物, 比如 3'-磷酸腺苷-5'-磷酸 (PAP) 以及赤藓糖磷酸酯合成中间产物 MEcPP 等, 也参与介导反馈信号调控植物光信号响应以及环境适应, 但是这些代谢物质在细胞核中的作用机制并不清楚^[95-98]。在转录后水平, 蛋白质组学数据显示叶绿素合成代谢酶 GSA-AT、CHLI、CHLH、POR、DVR 等均具有潜在的磷酸化位点^[99-100]。根据泛素化组学数据, 叶绿体内的很多蛋白存在泛素化修饰, RING 型 E3 泛素连接酶 SP1、CDC48 复合体、PUB4 等被证实介导叶绿体或叶绿体内蛋白质泛素化降解^[101-104]。叶绿素合成代谢酶进入叶绿体发挥功能依赖于叶绿体的跨膜转运复合体, 在莱茵藻中发现叶绿体基因组最大基因编码的蛋白 Orf2971 参与蛋白质转运和质量控制, 维持叶绿体稳态^[105]。同时, 我国两个实验室几乎同步解析了 TOC (叶绿体外膜上的转运子) 和 TIC (叶绿体内膜上的转运子) 相连形成的超级复合物, 解决了一直以来的争议, 为后续解析细胞核编码蛋白转运对叶绿素合成的调控奠定了重要的结构学基础^[106-107]。此外, 硫氧还蛋白 TRX (redox-active thioredoxins) 与 NTRC (NADPH dependent thioredoxin reductase C) 介导的氧化还原反应、一氧化氮介导的 S-亚硝基化 (S-nitrosylation) 修饰、蛋白质赖氨酸和精氨酸的甲基化修饰等均被证实在叶绿体四吡咯合成过程中发挥重要作用, 比如水稻 OsTRX 与 OsMORFs 互作调控叶绿体 RNA 编辑效率, 进而调控叶绿体发育^[14,108]。GLK1/GLK2 转录因子在叶绿体发育中非常重要, 我国研究团队将 C₄ 植物玉米 GLK 在 C₃ 水稻中表达可调控气孔闭合, 提高水稻的光合效率和耐旱性, 为利用 C₄ 植物基因改造 C₃ 植物提升光合效率提供了参考^[109-110]。

尽管这些较新的调控机制研究大多为组学数据或特例探究, 但是这些调控方式的发现及其重要性不言而喻。叶绿体反馈信号的复杂性、光信号与激素信号之间的互作调控以及叶绿体代谢产物与细胞核转录调控之间的关联性正逐渐被解析和关注, 利用当下的 AI 技术、多组学大数据联合分析、单细胞测序等先进研究手段深入探究这些调控机制和多信号间的互作关系, 对于更深层次地理解和应用光

合作用具有重要意义。

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