中国科学: 生命科学 2023 年 第 53 卷 第 9 期: 1213 ~ 1229
SCIENTIA SINICA Vitae lifecn.scichina.com
《中国科学》杂志社 SCIENCE CHINA PRESS

评 述

植物RuBisCO研究进展

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收稿日期: 2023-01-03; 接受日期: 2023-03-08; 网络版发表日期: 2023-08-30
国家重点研发计划项目(批准号: 2016YFD0300100)资助

摘要 提高光合作用效率是未来进一步提高作物产量和生物量的有效途径之一. 1,5-二磷酸核酮糖羧化酶/加氧酶(ribulose-1,5-bisphosphate carboxylase/oxygenase, RuBisCO)是光合碳同化过程中的关键酶, 催化RuBP与CO_2的羧化反应, 将无机碳固定为有机碳, 是提高植物光合作用效率的重要靶标. 然而, RuBisCO催化速率低且底物特异性差, 不能有效区分二氧化碳和氧气, 因此被称为"低效率酶". 鉴于RuBisCO在光合作用过程和全球碳循环中的重要作用, 以及在提升作物光合作用效率和产量方面具有广泛应用潜力, RuBisCO的遗传改造已成为光合作用领域研究的前沿热点并取得了重要进展. 本文系统综述了RuBisCO的分类进化、折叠组装机制、自然变异以及响应环境变化的生理生化机制, 重点介绍了RuBisCO遗传改造方面的研究进展, 并对RuBisCO未来研究趋势进行了展望和讨论, 以期为今后的研究提供重要借鉴和参考.

关键词 1,5-二磷酸核酮糖羧化酶/加氧酶, RuBisCO活化酶, 自然变异, 环境响应, RuBisCO遗传改造

研究预测, 到2050年, 全球人口预计将达98亿, 全球粮食产量需在2010年的基础上增加50%以上才能满足人口增长的需要[1]. 2020年新冠疫情的暴发加剧了世界饥饿和贫困现状. 联合国最新报告表明, 2021年全球饥饿人口达8.28亿, 占世界总人口的9.8%[2]. 因此, 未来需要持续提高作物产量来解决人类粮食刚性需求.

光合作用是几乎所有生命物质和能量的来源, 也是作物生物量和产量形成的基础. 研究表明, 提高作物光能利用效率和光合作用效率是未来大幅提高作物产量的关键而有效途径之一[3~7]. 1,5-二磷酸核酮糖羧化酶/加氧酶(ribulose-1,5-bisphosphate carboxylase/oxygenase, RuBisCO)是光合作用中卡尔文-本森循环(Calvin-Benson-Bassham cycle, CBB cycle)催化第一步反应的酶, 直接将无机CO_2转化为有机碳水化合物, 是决定光合碳同化效率的关键酶和限速酶[8,9]. 研究表明, 地球上每年有超过1000亿吨CO_2被转化为生物材料或有机化合物, 其中超过99%的有机物由RuBisCO固定产生[10~12]. 因此, RuBisCO在整个光合作用过程、生物质合成和全球碳循环中扮演着重要角色. 然而, RuBisCO催化速率低(3~20个CO_2/s)且底物特异性差, 不能有效区分CO_2和O_2[13]. 为了弥补其低催化速率的缺陷, 高等植物消耗大量的氮素来合成RuBisCO, RuBisCO含量约占叶片可溶性蛋白总量的10%~50%[14], 是地球上含量最丰富的蛋白[12~15].

引用格式: 周昱婕, 李霞, 陈根云, 等. 植物RuBisCO研究进展. 中国科学: 生命科学, 2023, 53: 1213–1229
Zhou Y J, Li X, Chen G Y, et al. Research progress in plant RuBisCO (in Chinese). Sci Sin Vitae, 2023, 53: 1213–1229, doi: 10.1360/SSV-2022-0275

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1 RuBisCO的发现历程

RuBisCO的发现可以追溯到76年前(图1). 1947年, Wildman和Bonner[16]利用硫酸铵分级方法首次从菠菜(*Spinacia oleracea*)叶片中分离出RuBisCO, 称其为"组分Ⅰ蛋白(fraction I protein)". 1954年, Quayle等人[17]发现, 小球藻(*Chlorella vulgaris*)光合作用的碳同化过程是1,5-二磷酸核酮糖(ribulose-1,5-bisphosphate, RuBP)与CO_2发生羧化反应, 生成2分子的磷酸甘油酸(3-phosphoglycerate, PGA). 1955年, Wilson和Calvin[18]在菠菜中制备了羧化酶系统, 确认是由一种"羧化酶"催化RuBP的羧化反应, 这种羧化酶即RuBisCO. 1971年, Bowes等人[19]进一步发现RuBisCO是一个双功能酶, 还能催化O_2与RuBP竞争性结合发生的加氧反应. 1982年, Somerville等人[20]通过分离拟南芥(*Arabidopsis thaliana*) *rca*突变体的叶绿体蛋白, 首次发现了RuBisCO活化酶(RuBisCO activase, Rca), 为揭示RuBisCO的体内活化机制奠定了基础. 1986~1988年, 研究者陆续解析了RuBisCO的折叠机制和三维结构[21~23]. 2014年, 研究人员通过质体转化技术, 将模式蓝细菌细长聚球藻(*Synechococcus elongatus*)PCC7942菌株的RuBisCO及分子伴侣替换了烟草(*Nicotiana tabacum*)自身的*NtrbcL*基因, 所得转化体可成功组装具有活性的RuBisCO全酶, 并且在高浓度CO_2条件下的羧化活性和CO_2固定效率均高于野生型[24]. 2017年, 通过在大肠杆菌(*Escherichia coli*)中共表达拟南芥RuBisCO大小亚基及多个叶绿体伴侣蛋白, 实现拟南芥RuBisCO在大肠杆菌中的表达, 并解析了植物RuBisCO的组装过程[25]. 随着研究的不断深入, 目前对RuBisCO的结构、功能及其调控机制等方面有着越来越全面的了解.

2 RuBisCO的分类和结构

RuBisCO全酶通常由大亚基(large subunit, LSU)(~52 kD)和小亚基(small subunit, SSU)(~16 kD)组成. 在植物中, 大小亚基分别由叶绿体基因*rbcL*和核基因*rbcS*编码. 在自然界, 根据立体结构的相似性以及氨基酸序列的同源性, RuBisCO分为Ⅰ型、Ⅰ′型、Ⅱ型、Ⅲ型、Ⅱ/Ⅲ型和Ⅳ型等6种类型(表1)[26~29]. 其中Ⅰ型最为丰富, 由八个大亚基和八个小亚基组成L_8S_8全酶, 分子量约为550 kD, 一般存在于真核生物和细菌中[26]. Ⅰ′型RuBisCO在绿弯菌门(Chloroflexi)中发现, 其LSU与Ⅰ型同源, 但缺少SSU[27~29]. Ⅱ型RuBisCO由1~2个大亚基组成, 结构上与Ⅰ型有25%~30%的相似性[33,34], 一般存在于变形菌(*Proteobacteria*)和甲藻(*Dinophyceae*)中[35]. Ⅲ型RuBisCO只存在于古细菌中, 由3~5个大亚基构成[36~38]. Ⅱ/Ⅲ型在伯氏甲烷球菌(*Methanococcoides burtonii*)中被发现, 最初称其为MBR(*M. burtonii* RuBisCO). 氨基酸序列分析表明, MBR的开放阅读框起源于Ⅱ型RuBisCO, 但在构型上, MBR的催化活性位点却又与Ⅲ型相近, 因此MBR属于特有的Ⅱ/Ⅲ亚型[39]. Ⅳ型, 也称RuBisCO类蛋白(RuBisCO-like protein, RLP), 从绿色硫磺细菌(*Chlorobium tepidum*)中克隆而来, 其序列与RuBisCO相似, 但缺少催化位点, 不具备RuBisCO的催化功能[40].

通过序列比对以及晶体结构解析表明, RuBisCO大亚基在各种亚型中的氨基酸序列以及结构相似性较高[41]. 大亚基N端通常为一个约150个残基的α/β结构域, C端包含一个约310个残基的$\beta_8\alpha_8$ 磷酸丙糖异构酶(triose-phosphate isomerase, TIM)-桶形结构域和一个约15个残基的柔性尾巴[31], 每个大亚基都具有一个催化活性位点和一个调节位点. 大亚基C端332~338残基

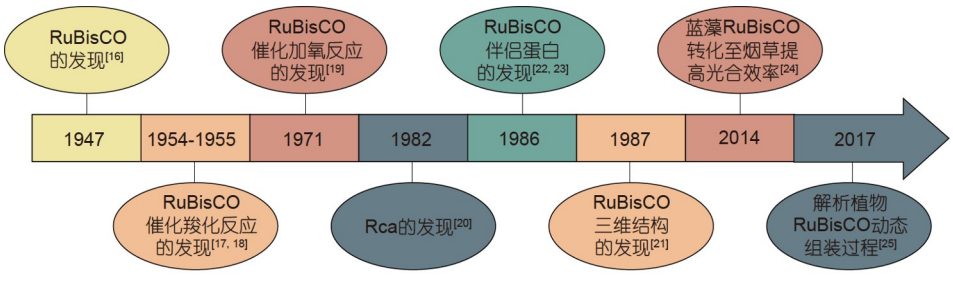

图 1 RuBisCO的研究历程
Figure 1 The timeline of RuBisCO research

表 1　RuBisCO的分类

Table 1　The classification of RuBisCO

RuBisCO类型		结构特点	存在物种	参考文献
Ⅰ型	IA	L_8S_8	变形菌、蓝藻	[30,34]
	IB	L_8S_8	高等植物	[34]
	IC	L_8S_8	细菌、变形菌	[34,36]
	ID	L_8S_8	非绿藻	[31,34,36]
Ⅰ′型	–	L_8	绿弯菌门	[27~29]
Ⅱ型	–	$(L_2)_n$	变形菌、甲藻	[33~35]
Ⅲ型	–	$(L_2)_n$	古细菌	[32,37,38]
Ⅱ/Ⅲ型	–	$(L_2)_n$	伯式甲烷球菌	[39]
Ⅳ型(RLP)	–	$(L_2)_n$	细菌、古细菌、藻类	[39]

构成的环状结构(loop 6)可在RuBisCO活性状态转变过程中灵活运动，对于活性位点的开闭是必需的，同时C末端在此过程中保持结构的稳定. 另外，Lys201(植物RuBisCO氨基酸序列编号，第201位赖氨酸)发生氨基甲酰化修饰后与Mg^{2+}结合，这个过程对于正确结合底物RuBP是必需的[31,41].

研究发现，RuBisCO小亚基虽不直接参与形成活性位点，但能够稳定全酶结构，是RuBisCO实现催化活性所必需的[42,43]. 与大亚基的相对保守不同, RuBisCO小亚基的序列更具有多样性, 其共同的核心结构由四条反向平行的β折叠组成，在其一侧被两个α螺旋覆盖[44]. 不同物种间小亚基的变异主要发生在βA-βB环(βA-βB loop，即连接β折叠A和β折叠B之间的氨基酸环)之间以及C末端结构中. 小亚基的进化被认为是对大气中不断增加的氧气水平的适应，因此小亚基对Ⅰ型RuBisCO的CO_2/O_2特异性结合(RuBisCO CO_2/O_2 specificity, $S_{c/o}$)起重要作用, 通常缺少小亚基的RuBisCO $S_{c/o}$值很低[45,46]. 最新的研究利用“祖先序列重建”(ancestral sequence reconstruction)技术重演了Ⅰ型RuBisCO的进化过程，结果表明早在大气O_2浓度升高之前，Ⅰ型RuBisCO就已获得了小亚基，并迅速成为决定RuBisCO催化活性和特异性的必要组分[27].

3　RuBisCO的表达、折叠与组装

RuBisCO的生物合成包括基因表达、蛋白折叠、成熟蛋白的组装等多个过程，而编码基因*rbcL*和*rbcS*的表达同样受到转录、转录后及翻译等多个水平的调控. 在转录水平上，*rbcS*启动子中包含光响应元件(GT元件等)，受光诱导表达[47]. *rbcL*主要受转录后水平的调控[48]，有研究发现RLSB(核编码的S1结构域RNA结合蛋白)是C_3和C_4植物中*rbcL*转录后调控因子，特异性调控*rbcL*在C_4维管束鞘细胞中的表达[49]; Mrl1(核编码的五肽重复序列蛋白)在衣藻(*Chlamydomonas*)和拟南芥中结合*rbcL* mRNA并促进其加工[50]. 此外, RuBisCO大小亚基还受到翻译后水平的调控，维管植物中大多存在LSU氨基甲酰化、脱甲酰化、乙酰化、甲基化和N端蛋白水解，以及SSU转运序列处理等翻译后调控机制，但具体的生化功能尚不了解[51,52]. 衣藻和高等植物叶绿体中的上位性合成机制(control by epistasis of synthesis, CES)也可调控叶绿体编码大亚基的翻译速率，在SSU缺乏的情况下，CES导致LSU合成明显减少[53]. Wietrzynski等人[54]在衣藻中进一步发现，缺少SSU时，LSU_8-RAF1复合物会对LSU的翻译产生负反馈调节, 进而调控RuBisCO全酶的组装.

分子伴侣是存在于细菌或真核细胞中的一类蛋白，能够在正常或胁迫状态下结合未折叠的蛋白疏水残基，协助蛋白质的正确折叠，防止错误折叠或聚集[55,56]. RuBisCO大小亚基需要分子伴侣蛋白及其辅助因子的帮助才能进行正确折叠和组装，形成完整的全酶结构. RuBisCO与分子伴侣蛋白在进化过程中存在协同进化的关系，不同亚型RuBisCO的分子伴侣蛋白存在差异[57]. 原核生物中RuBisCO的分子伴侣蛋白为GroEL(growth essential large), 真核生物叶绿体中为Cpn60(chaperonin 60)；所对应的辅伴侣蛋白(co-chaperonin)分别为GroES(growth essential small), Cpn10/

Cpn20(chaperonin 10/20).

GroEL/GroES是被广泛研究的分子伴侣，最早发现于大肠杆菌突变体*groE*，它能够特异阻断噬菌体的头部装配[58]，GroEL/GroES是GroE的同源蛋白，被认为是原核细胞负责翻译后辅助折叠和组装的必要因子[22]. 通常在细菌中, RuBisCO大亚基的折叠由GroEL/GroES完成，而小亚基能够自发折叠并与大亚基组装形成全酶，这其中还需要其他辅助因子的作用. 而在植物中，RuBisCO的折叠组装依赖于与GroEL同源的叶绿体分子伴侣Cpn60α和Cpn60β，其中Cpn60为十四亚基复合体，通常由β同源寡聚体或α和β异源寡聚体以1:1的比例组成[59]. 将莱茵衣藻(*Chlamydomonas reinhardtii*)的Cpn60α和Cpn60β在大肠杆菌中共表达可替代GroEL，说明叶绿体伴侣蛋白的功能与GroEL/GroES类似[60].

植物RuBisCO的组装不仅依赖于Cpn60对大亚基的折叠, 还需要其他伴侣蛋白和辅助因子的参与. 目前已知参与RuBisCO组装的辅助因子有RbcX，Raf1(RuBisCO assembly factor 1)，Raf2(RuBisCO assembly factor 2)以及BSD2(bundle sheath defective 2)等，其中RbcX，Raf1与RbcL相互作用，Raf2的功能尚不明确[25,61~68]. 这些辅助因子与RuBisCO亚基的相互作用本质上是动态变化的，且结合过程不依赖于ATP. 研究者将拟南芥的RuBisCO大小亚基、分子伴侣和辅助因子转化至大肠杆菌以探究RuBisCO的组装机制，发现新合成的RbcL亚基被Cpn60αβ/Cpn20伴侣蛋白折叠后，先与Raf1或RbcX平行组装成二聚体$RbcL_2$Raf1或$RbcL_2$$RbcX_2$，继而BSD2与Raf1/RbcX/Raf2置换形成$RbcL_8$，并进一步稳定多聚体结构；最后RbcS置换BSD2后与$RbcL_8$结合，形成$RbcL_8RbcS_8$功能性全酶[25]. 有研究利用单颗粒冷冻电子显微镜技术解析了蓝藻RuBisCO及分子伴侣Raf1和RbcX组装过程的多层次动态构象，为揭示RuBisCO组装的分子机制奠定了基础[69,70].

总体而言，不同物种中RuBisCO的折叠和组装机制有所差别，且伴侣蛋白、辅助因子与RuBisCO之间存在协同进化关系，因此深入解析RuBisCO组装过程，有利于在高等植物中构建异源高效RuBisCO全酶.

4 RuBisCO的自然遗传变异

自然界中RuBisCO具有多样性，明确不同物种间RuBisCO的遗传变异，比较不同RuBisCO的酶动力学性质，对于培育具有高催化效率RuBisCO的作物新品种具有重要指导意义[71~76].

Orr等人[77]研究了不同温度条件下75个物种RuBisCO的催化特性，发现不同物种间RuBisCO的催化性能及大亚基序列存在多态性，并预测不同的氨基酸位点对羧化速率(k_{cat}^c)、$S_{c/o}$以及CO_2亲和力的贡献不同. Hermida-Carrera等人[78]分析了20种作物在三种不同温度下(15，25和35℃)的RuBisCO动力学曲线，结合*rbcL*，*matK*和*ndhF*基因的系统进化分析，发现RuBisCO大亚基中10个氨基酸残基发生了适应性进化. 针对C_3和C_4物种的比较表明，RuBisCO在不同环境条件下表现出催化特性的多样性[68,79~81]. 为了探究C_4光合作用的进化是否改变了*rbcL*的选择压力，Christin等人[82]对C_3和C_4植物*rbcL*密码子进行分析, 发现C_4植物RuBisCO的选择压力已被光合细胞中高CO_2环境所改变, 其中正向进化所选择的8个氨基酸可能是导致C_4植物RuBisCO具有更快羧化速率的原因. Sharwood等人[71]在10~37℃温度下检测黍族(*Paniceae*)C_3和C_4物种的RuBisCO动力学响应，结果表明，NADP依赖的苹果酸酶(NADP-Malic enzyme，NADP-ME)和磷酸烯醇丙酮酸羧激酶(phosphoenolpyruvate carboxykinase，PCK)光合途径中的RuBisCO羧化速率大于NAD依赖的苹果酸酶(NAD-Malic enzyme，NAD-ME)途径和C_3 RuBisCO，并且C_4大亚基Ser328和Glu470氨基酸的变化可能是导致RuBisCO催化能力存在差异的原因. 在同一物种间，RuBisCO催化特性也存在差异. Prins等人[75]研究了25个小麦(*Triticum aestivum*)品种和野生近缘种的催化特性，并结合其大亚基序列多样性进行分析，推测近缘种RuBisCO替代原生小麦RuBisCO能够提高小麦光合速率.

虽然RuBisCO主要的催化位点位于大亚基上(表2)，但最近越来越多的研究认为RuBisCO催化特性的差异可能源自远离活性位点的小亚基[41]，目前已发现的关键位点位于小亚基βA-βB loop和羧基末端[45]. 四个小亚基的βA-βB loop在全酶溶剂通道开口处排列，其中原核生物和非绿藻RuBisCO在βA-βB loop中只有10个氨基酸残基，高等植物中有22个，绿色藻类有28个[44,85,86]. 最新研究通过系统生物学方法预测了茄科植物进化过程关键时期的98种RuBisCO酶大小亚基的氨基酸编码序列，分析发现小亚基的序列变异比大亚基更多，且古老RuBisCO酶比现代RuBisCO催化效率

表 2　RuBisCO大亚基上影响催化活性的主要氨基酸位点

Table 2　The main amino acid sites affecting catalytic activity on RbcL

位点	位置	作用	参考文献
Lys201	loop2	RuBisCO发挥功能前进行甲氨酰化所必需的	[83]
Lys175	loop1	介导RuBP烯醇化	[83]
Lys334	loop6的顶端	催化过程中与气体底物相互作用	[44]
Asp203 Glu204	loop2	金属配体	[84]
His292 His294 His325 His327	C-terminal	有助于氨基甲酸酯基团上双负电荷的稳定	[84]

更高，推测祖先酶的小亚基可能对全酶的催化动力学产生积极的影响[87]. Matsumura等人[88]发现，水稻(*Oryza sativa*)RbcL与高粱(*Sorghum bicolor*)RbcS组装的杂交型RuBisCO催化效率(k_{cat})显著高于野生型水稻，表明小亚基氨基酸变化对RuBisCO动力学具有重要影响. 此外，将烟草叶片腺毛细胞中存在的一种RbcS亚型(NtRbcS-T)转化至莱茵衣藻中，发现得到的杂合RuBisCO具有更高的最大羧化效率(maximum carboxylation rate，V_{cmax})和K_m[89~91]. 将马铃薯(*Solanum tuberosum*)*StrbcL*和*StrbcS-T*转入已沉默自身*rbcS*基因的烟草材料(tobRr$^{\Delta S}$)中，所得转化植株RuBisCO的k_{cat}^c值增加，但对CO_2亲和力降低，导致羧化效率和光合效率降低，而通过在氨基酸水平对小亚基进行改造可提高其羧化效率，进一步说明RuBisCO小亚基对于催化活性以及光合效率具有重要贡献[92].

5　RuBisCO对不同环境因子的响应

RuBisCO活性直接受环境变化的影响，不同环境条件下，尤其是高温、干旱和低温等逆境条件，常会导致作物光合速率下降，其中RuBisCO活性降低是引起光合速率下降的重要原因之一. 提高环境胁迫条件下RuBisCO的羧化效率对于提高作物光合速率以及胁迫耐受能力，进而提高作物产量具有重要的意义[93,94].

5.1　RuBisCO对温度的响应

RuBisCO动力学性质对温度极为敏感[95,96]，但由于每个动力学参数的温度依赖性不同，对于给定温度变化的相对影响是不同的[97]. 随着温度的增加，RuBisCO的$S_{c/o}$降低，使得RuBisCO加氧反应增强，导致光呼吸加剧，造成能量损耗[98]. 此外，C_3和C_4植物RuBisCO对于温度的响应存在差异. 比较17种C_3和C_4植物的RuBisCO催化效率表明，C_4植物和起源于凉爽环境的C_3植物的RuBisCO具有较高的k_{cat}，而起源于温暖环境的C_3植物k_{cat}较低. C_4植物和起源于凉爽环境的C_3植物中，RuBisCO经常在接近CO_2饱和的情况下运行，因此k_{cat}的增加会提高CO_2同化率；而起源于温暖环境的C_3植物，RuBisCO经常在低于K_m的CO_2浓度下运行，由于k_{cat}和K_m成比例地变化，较低的k_{cat}表示RuBisCO的K_m值降低，因此温暖气候下C_3植物对CO_2的亲和力增加[99]. Sharwood等人[71]分析了不同植物RuBisCO对于温度变化的响应，发现在25℃前后，植物RuBisCO的k_{cat}^c值表现出双线性响应；与NAD-ME和C_3/C_2物种RuBisCO相比较，随着温度的增加，NADP-ME和PCK型植物的RuBisCO具有更高的k_{cat}^c和K_c(CO_2的米氏常数)，而$S_{c/o}$下降较慢，这同样与它们的起源相关. 利用不同物种RuBisCO对温度变化的响应特性不同，从而进行优化，可使作物更好地应对未来环境温度的挑战[100]. 值得注意的是，RuBisCO含量同样影响植物对温度的响应，研究发现，增加RuBisCO含量能使玉米(*Zea mays*)更好地应对低温胁迫，并改善玉米遭遇低温后的恢复能力[101].

RuBisCO的活性也受到磷酸糖等RuBP类似物的限制，阻碍其与底物的结合. 为了恢复RuBisCO的催化能力，需要Rca从活性位点去除抑制性化合物. Rca是AAA$^+$(ATPases associated with various cellular activities)蛋白家族的成员，利用ATP水解产生的能量促进磷酸化抑制剂的释放[102,103]. Rca对温度十分敏感，当

温度高于光合作用的最适温度时, Rca活性和含量均受到抑制. Degen等人[104]使用定点诱变, 发现小麦Rca的Met159被异亮氨酸取代后, 可将Rca的最适温度提高5℃, 同时保持Rca活化RuBisCO的效率不变. 在*rbcS*过表达株系中过表达*Rca*不仅能够恢复RuBisCO活化状态, 还能提高在高温下(32~36℃)转基因水稻的CO_2同化效率[105~108]. 将*ZmRca*与*OsrbcS2*在水稻中共同过表达, 能够维持水稻在高温下(40℃)的光合效率[109]. 以上研究表明, 在未来全球气候变化加剧的情况下, 深入研究RuBisCO和Rca间的协同作用将为提高作物光合效率和产量提供有效策略.

5.2 RuBisCO对CO_2浓度上升的响应

C_3植物中, RuBisCO与CO_2的反应是不饱和的, 因此会发生竞争性加氧反应导致光呼吸. 理论上, 短期CO_2浓度升高可能提高RuBisCO羧化速率, 同时减少O_2对CO_2在RuBisCO活性位点的竞争, 降低光呼吸, 从而提高C_3植物的光合效率[110~113]. 对C_3作物水稻的研究发现, 不同品种之间的种间变异对高CO_2环境的产量响应不同. Zhu等人[114]对两个水稻品种五香粳14和汕优63进行开放式空气CO_2浓度增加(free-air CO_2 enrichment, FACE)实验, 测定其产量和光合生理指标后发现, 在高CO_2浓度下, 汕优63较五香粳14具有更高的RuBisCO含量、光合速率和产量, 这可能与汕优63水稻“源”的增强以及在籽粒发育期间仍维持较高的光合能力有关. 扬稻6号和武运粳23水稻的FACE实验显示, 在较高的CO_2浓度下, 扬稻6号较武运粳23具有更高的光合速率、叶片RuBisCO含量、V_{cmax}和最大电子传递效率(maximum electron transport rate, J_{max}), 同时其硝酸还原酶(nitrate reductase, NR)、谷氨酸合成酶(glutamate synthase, GOGAT)活性和叶绿素含量也显著增加, 产量也有明显提高[115].

另一方面, 有研究发现, 植物长期处于高CO_2环境可能会降低光合效率, 主要原因为碳水化合物过量积累或与氮含量降低有关的次级反应, 降低了RuBisCO活性、电子传递速率和气孔导度[116~118]. 由于CO_2增加往往伴随着高温干旱等一系列胁迫环境的出现, 未来作物如何适应环境可能还需要进一步探索.

5.3 RuBisCO对光的响应

自然界中植物时刻处在光的波动环境下. *rbcS*基因的表达受光诱导, 通常在光下*rbcS*表达量增加, 这由其启动子区的光敏元件控制[119,120]. RuBisCO大亚基基因*rbcL*的表达量也受光照影响, Yerramsetty等人[121]认为, *rbcL* mRNA结合蛋白RLSB受光诱导参与*rbcL*转录后调控, 从而影响RuBisCO的合成.

此外, RuBisCO活性也依赖于光的调节[122], 其活化状态受到催化位点的氨甲酰化、磷酸糖的抑制和Rca的激活等动态调控. 氨甲酰化取决于叶绿体基质的pH值、CO_2和Mg^{2+}浓度[123,124], 其中叶绿体基质的pH值只有在光照下才能达到氨甲酰化所需的最优值[125,126]. RuBisCO的催化速率还受到底物RuBP浓度的调节, 在光照下RuBP浓度达到饱和, 而黑暗或弱光时, RuBP下降到不饱和水平, 此时某些磷酸糖抑制物与RuBisCO的结合将会抑制其活性[127,128]. 此外, 体内RuBisCO的活性必须由Rca激活, 而Rca具有光强依赖性[129,130]. Zhang等人[131]发现, 不同光强下叶绿体基质的氧化还原状态可以调节Rca活性, 从而导致低光下RuBisCO活性下降. 进一步研究发现, 拟南芥中Rca存在氧化还原不敏感的亚型, 该亚型Rca在从低光过渡到高光后, 其诱导CO_2同化的速度显著提高, 且在波动光条件下具有更快的光合诱导效率, 表明通过调控Rca可能增强植物在动态光环境下的光合能力[128].

5.4 RuBisCO对氮素的响应

氮素是植物生长发育必需的营养元素之一, 是蛋白质的主要组成元素[132]. RuBisCO占叶片总氮的15%~30%, 是植物体内重要的氮素来源, 参与植物的生长发育过程[133]. 在水稻、小麦等作物灌浆期, 剑叶和穗部的RuBisCO作为氮源被降解为氨基酸, 转移至籽粒参与灌浆过程[134~136]. 水稻营养生长过程中, 衰老叶片RuBisCO经自噬系统降解, 将氮素重新分配到其他幼叶或器官中进而参与植物生长[137].

氮素的缺乏直接影响RuBisCO的合成与调控. 研究表明, 光合作用的限制因素可能受叶片氮含量的影响, 低氮时, 光合效率受RuBisCO羧化效率的影响; 高氮时, 光合效率受RuBP再生速率的限制[138,139]. 长期缺氮时, RuBisCO含量会显著下降, 导致RuBisCO活性和净光合速率下降; 但短期氮缺乏情况下, Rca活性增加可促进RuBisCO活化, 提高氮素利用率, 促进光合作用[140]. 在高氮条件下, 增加的RuBisCO含量主要起到储存氮素的作用, 而并非增加催化活性, 最终由于CO_2

供应不足导致RuBisCO活性降低, 氮素利用率下降, 光合效率下降[141]; 但高氮下储存的RuBisCO可以在逆境胁迫和衰老过程中介导氮素的重新分配[133].

一些研究认为, 光合作用的反馈抑制与叶片碳氮比有关[142,143]. 在氮缺乏条件下, 过量的碳水化合物积累在叶片中[138], 其中磷酸糖会结合在RuBisCO的活性位点, 从而导致光合作用的反馈抑制[144]. 而Rca活性增加可以解除磷酸糖对RuBisCO活性的抑制, 有助于改善RuBisCO羧化能力. 因此提高光合产物的转运效率和RuBisCO的活化能力是建立作物耐低氮机制的有效途径之一.

6　RuBisCO的遗传改造

自然界中不同光合生物的RuBisCO活性和羧化效率(活性通常指RuBisCO催化反应的能力, 羧化效率则指RuBisCO催化羧化反应的能力)等均存在很大差异. 相较于C_4作物而言, C_3作物的RuBisCO是一个低效酶, 理论上提高C_3作物RuBisCO活性, 增加羧化效率可有效提高其光合作用效率. 目前对于RuBisCO的改造主要集中在以下两个方面: RuBisCO异源重组改造和RuBisCO编码基因的定点突变(图2).

6.1　RuBisCO异源重组改造

通过叶绿体转化等技术将不同植物来源的RuBisCO亚基进行异源组装是改善作物RuBisCO羧化特性的现有策略之一[145]. Zhu等人[112]通过模拟计算表明, 用一种凋毛藻属(*Griffithsia monilis*)RuBisCO替代现有C_3作物RuBisCO可以使有机物积累增加超过25%. 利用系统模型预测发现, 将类黍尾稃草(*Urochloa panicoide*)RuBisCO转至小麦和玉米, 能提高它们的碳同化效率[146].

高等植物中, 受质体转化技术的限制, 外源RuBisCO的异源重组改造主要在烟草中进行. 1999年, 研究人员通过质体转化分别将向日葵(*Helianthus annuus*)和蓝藻(*Synechococcus*)PCC6301的*rbcL*基因导入至烟草, 成功得到了具有活性的向日葵大亚基和烟草小亚基异源重组的嵌合酶, 但转基因烟草无法在正常CO_2浓度下生长[147]. 2001年, Whitney和Andrews[148]将深红红螺菌(*Rhodospirillum rubrum*)来源的II型RuBisCO

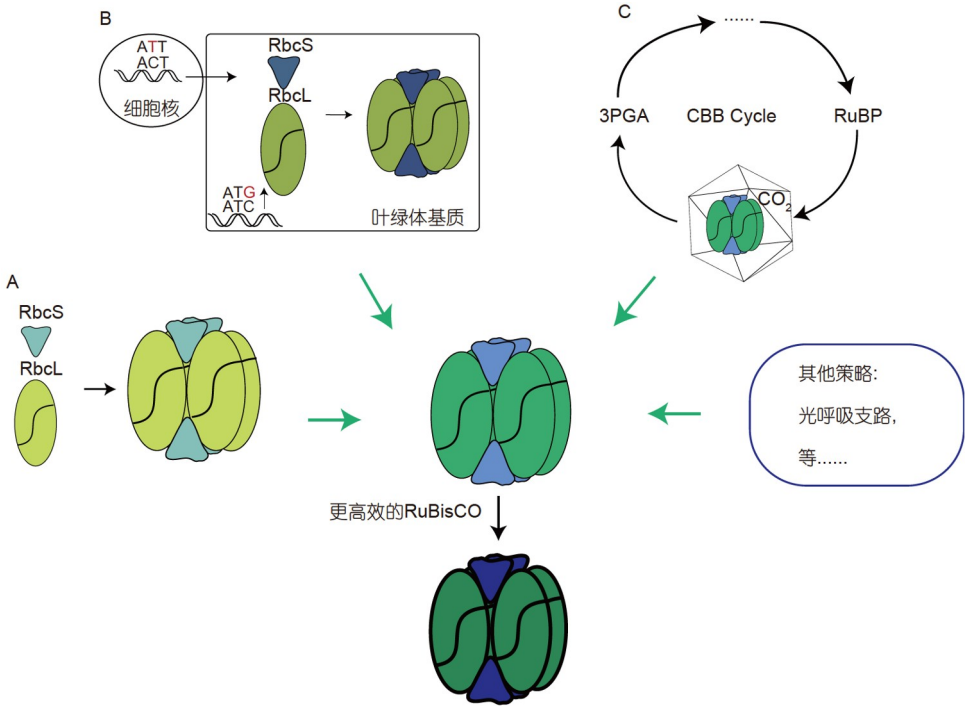

图 2　提高RuBisCO羧化效率的主要方法. A: RuBisCO的重组改造; B: RuBisCO编码基因的定点突变; C: 在植物中构建CCM

Figure 2　The main methods to improve the efficiency of RuBisCO carboxylation. A: The restructuring and transformation of RuBisCO; B: site-directed mutation of the RuBisCO encoding gene; C: building CCM in plants

(RbcM)转化至烟草，其RuBisCO动力学特性与深红红螺菌的一致，转基因植株能够在高CO_2浓度下自养和繁殖，这表明异源重组RuBisCO能够维持叶绿体的光合代谢. 此外，将黄顶菊(*Flaveria bidentis*)的*rbcL*转化至烟草，其转化植株RuBisCO的K_c和V_c均显著高于烟草，与*F. bidentis* RuBisCO特性相符[149].

目前，虽然能在烟草中表达外源RuBisCO，但多数转化体植株均伴随着RuBisCO含量降低、光合能力下降和生物量减少等问题(表3). 对水稻RuBisCO改造中发现，用高粱小亚基替换水稻小亚基，产生杂合的L_8S_8，其催化活性显著提高. 晶体结构分析发现，SbRbcS中的Leu102可以促进RuBisCO催化活性并改变其动力学性能，但转基因植株中RuBisCO含量显著低于野生型，因此杂合RuBisCO水稻生长情况没有显著变化[88]. Sharwood等人[150]通过对Kanevski等人[147]构建的杂合RuBisCO烟草材料tobacco^{Rst}进一步研究，发现tobacco^{Rst}虽能在高CO_2浓度下自养和繁殖，但生长速度仅为野生型烟草的1/4，进一步研究发现tobacco^{Rst}中RuBisCO含量和*rbcL*/*S* mRNA水平明显降低，推测限制烟草生长的主要原因是异源大小亚基的翻译和/或组装机制不相容. 另外，与质体合成的异源小亚基相比，核编码的小亚基优先参与L_8S_8组装，同样也是杂合RuBisCO组装的阻碍[148].

基于异源RuBisCO体内重组存在障碍，研究者们尝试利用共转化异源RuBisCO的组装辅助因子来解决折叠组装问题. Whitney等人[151]将拟南芥*rbcL*和*Raf1*基因共转化至烟草时发现，共转化植株较*rbcL*转基因植株的RuBisCO含量增加，生长速率更快，光合速率更高. 在玉米中同时过表达自身*RbcL*，*RbcS*和*Raf1*基因，RuBisCO的含量增加30%以上，转基因植物的CO_2同化能力增加15%[152]. 研究发现，球形红细菌(*Rhodobacter sphaeroides*)中的*Rs*RuBisCO在植物叶绿体中组装不存在折叠障碍，与蓝藻L_8S_8在烟草中的表达相比，*Rs*Rubisco的产量高出3倍以上. 共表达*Rs*RuBisCO和*Rs*Rca可以提升RuBisCO的活性，并增加烟草的光合作用效率和生长速率[153]. Chen等人[154]最新研究发现，硫氧菌(*Halothiobacillus neapolitanus*)来源高速RuBisCO在烟草叶绿体中的表达与组装不依赖任何额外的伴侣蛋白，RuBisCO产量可以达到野生型的40%左右，转基因植株在1% CO_2下表现出和野生型一样的生长速率. 该研究中RuBisCO的高效组装为后续细菌CO_2浓缩机制(CO_2 concentrating mechanism, CCM)的利用打下良好基础. 因此，对于RuBisCO的重组改造，不仅要考虑大小亚基、RuBisCO和Rca之间的互作，还应该考虑分子伴侣蛋白和组装辅助因子的特异性贡献，并寻找高效组装的RuBisCO类型，从而解决不同物种叶绿体折叠组装机制不相容而造成RuBisCO蛋白含量或者活性不足的问题.

另一方面，通过异源重组RuBisCO，还能够将外源CCM构建至高等植物叶绿体内，进一步提高RuBisCO的羧化能力. CCM是指在C_4植物、衣藻和蓝藻等生物中存在一种机制，能利用一些特殊的结构和酶，将CO_2浓缩至RuBisCO周围，从而提高其羧化效率. 目前在C_3植物中构建蓝藻CCM已获得部分进展. Lin等人[24,155]

表 3 RuBisCO异源重组改造后含量和动力学特性的变化[a)]

Table 3 The changes of the content and kinetic properties of RuBisCO after non homologous recombination[a)]

材料名称	组装类型	RuBisCO含量	k_{cat}^c	$S_{c/o}$	$K_c^{21\%O2}$	生物量	参考文献
CSS16, SS10	水稻大亚基与高粱小亚基组装	*↓	–	*↓	*↑	*↓	[88]
tob_{pLpS1}	马铃薯不同类型小亚基与大亚基分别组装至烟草	*↓	–	*↑	–	*↓	[92]
tob_{pLpS2}		–	–	*↑	–	*↓	
tob_{pLpS3}		–	*↑	*↑	–	*↓	
tob_{pLpST}		–	*↑	*↓	*↑	*↓	
SeLSX	蓝藻大小亚基及RbcX或CcmM35组装至烟草	*↓	*↑	–	–	–	[155]
SeLSM35		*↓	*↑	–	–	–	
tobRsLS	球形红细菌大小亚基及RbcX组装至烟草	*↓	*↑	*↓	*↑	–	[153]
tobRsLS::X		*↓	*↑	*↓	*↑	–	
*Hn*RuBisCO	硫氧菌大小亚基转化至烟草	*↓	*↑	–	–	–	[154]

a) *表示显著性，↓表示减少，↑表示增加，–表示没有数据

实现了在烟草叶绿体中表达β-羧酶体外壳和IB型RuBisCO, 这是在植物中装配羧酶体的第一步. Long等人[156]将蓝藻的IA型RuBisCO大、小亚基基因, 壳蛋白和linker蛋白基因替换烟草*rbcL*, 成功构建了简化版羧酶体, 所得转化体在高浓度CO_2下能够自养生长, 这为构建具备CCM的作物奠定了基础.

6.2　RuBisCO编码基因的定向突变

早期研究通常以衣藻作为材料, 通过分析其突变体的RuBisCO活性或$S_{c/o}$, 选择性地将有利突变引入到烟草中, 以期改变烟草RuBisCO动力学特性[4]. Whitney等人[149]对烟草RbcL进行Q149A和V265I突变后, RbcL和L_8S_8全酶蛋白量高于野生型, 且$tob^{flo\text{-}bid}$和tob^{flo}(转质体烟草, 大亚基序列中包含Asp149和Ile265)植株中RuBisCO含量显著高于$tob^{bid\text{-}flo}$和tob^{bid}(转质体烟草, 大亚基序列中包含Ala149和Ile265)植株, 推测149位氨基酸突变会影响RuBisCO的表达. Lin等人[157]使用特定的叶绿体转化系统将烟草*rbcL*进行大量单碱基突变, 获得多个RbcL稳定点突变株系, 并测定突变体的表型和生理指标, 以评估RbcL点突变对RuBisCO含量和活性的影响. 目前, 借助于CRISPR/Cas技术的发展, 可以对多个物种小亚基进行碱基编辑[158], 对于个别物种(如烟草), 能够对RuBisCO大小亚基编码基因分别进行点突变, 寻找结构中重要的氨基酸位点, 来创建具有最佳性能的植物RuBisCO.

7　展望

RuBisCO是光合碳同化过程的限速酶, 提高RuBisCO羧化效率对提高光合作用具有重要意义. 尽管RuBisCO作用关键, 但其催化效率低, 在植物中, 每一催化位点每秒仅完成2~5次羧化反应[153]. 此外, CO_2的固定不仅催化过程复杂, 且与O_2竞争, 造成能量浪费[112]. 因此, 通过改造RuBisCO来改善光合作用, 是未来协同提高作物产量和资源利用效率的重要途径之一. 此外, 提取和加工植物RuBisCO作为食物蛋白的相关研究正在兴起, 进一步为保障粮食安全提供新的方向[159].

近年来, 以CRISPR/Cas为代表的基因编辑技术得到快速发展, 为植物遗传改良提供了新思路[160]. 例如, 通过对水稻和玉米中参与淀粉合成的关键基因*Waxy*进行多位点编辑, 实现对籽粒直链淀粉含量的精细调控, 以选育优质的作物品种[161~164]. 基因编辑技术在RuBisCO研究中已有尝试, Khumsupan等人[158]使用CRISPR/Cas9体系和T-DNA插入系, 在拟南芥中构建了一系列*rbcS*家族单基因和多基因敲除突变体, 表明CRISPR/Cas系统是探索小亚基功能的可行方法. 随着基因编辑技术的日渐成熟, 目前已经能对植物质体基因进行有效编辑[165,166]. 以上研究为实现RuBisCO编码基因的单碱基定向精准编辑提供了可行基础, 有助于探明调控RuBisCO催化活性的分子机制, 并通过对RuBisCO的改造进一步优化其催化性能.

有研究指出, RuBisCO在物种间和物种内均存在巨大的序列差异, 且与其动力学特性相关, 因此利用基因组测序, 结合基因编辑和叶绿体转化技术, 对包括RuBisCO在内的光合相关酶进行选择性变异, 并应用机器学习预测RuBisCO动力学特性[167], 将为提高光合效率进而解决粮食生产问题提供新途径[168]. 未来还需要在以下几个方面需要进一步深入研究: (ⅰ) RuBisCO小亚基主要负责稳定全酶结构, 其结构变异对于RuBisCO的稳定性和催化活性产生影响, 但目前尚不清楚小亚基上哪些氨基酸位点起关键作用, 其作用机制及其调控机制还不甚清晰; (ⅱ) 作物中*rbcS*基因通常具有多个拷贝, 如何通过对小亚基基因的遗传操作解析各小亚基基因的生理功能差异, 也是今后亟待解决的科学问题和技术难点; (ⅲ) 应用CRISPR/Cas技术对RuBisCO基因进行单、多基因敲除, 以及单、多基因的定点突变, 能够产生全新的RuBisCO, 但这一过程是否需要Rca, Raf1/2和其他分子伴侣蛋白或辅助因子进行相应的改变目前尚未可知; (ⅳ) 过表达*OsrbcS2*能够提高水稻产量和氮素利用率[169]; *OsrbcS2*-RNAi, *OsrbcS3*-RNAi, *OsrbcS5*-RNAi株系的RuBisCO含量小幅降低, 但在高CO_2环境下水稻氮素利用率和生物量则有所增加[170], 这表明植物体内RuBisCO含量与碳-氮平衡间存在某种联系, 但目前尚不明确具体机制; (ⅴ) 目前RuBisCO的异源重组仅在烟草中进行, 而作物中只能过表达*rbcS*和*Rca*基因(表4), 难以有效增加RuBisCO活性和含量, 因此未来需要探索如何将优异RuBisCO重组到作物中以提高其光合能力和产量; (ⅵ)构建异源RuBisCO在高等植物中表达体系, 提升异源高速RuBisCO在高等植物中的表达与正确组装, 突破多基因叶绿体转化与表达技术, 成功

表 4 过表达*Rca*或*rbcS*基因对Rca或RuBisCO含量和活性的影响[a)]

Table 4 The effects of overexpressing *Rca* or *RbcS* gene on the content and activity of Rca or RuBisCO[a)]

材料名称	遗传类型	RuBisCO含量	RuBisCO活性	Rca含量	产量	参考文献
MRca5, MRca6	玉米*Rca*基因过表达至水稻	**↓	*↑	*↑	–	[105]
BRca5, BRca6	大麦*Rca*基因过表达至水稻	*↓	–	*↑	–	
OX-mRca	玉米*Rca*基因过表达至水稻	*↓	–	*↑	–	[171]
RBCS-sense	过表达水稻*rbcS*基因	*↑	*↓	–	↑	[169,172,173]
SS10, SS5	水稻大亚基与高粱小亚基组装	*↑	*↓	–	–	[174]
Pro_{RBCS}, Pro_{RCA}	在水稻中分别用*rbcS*启动子和*Rca*启动子过表达*Rca*	*↓	*↑	*↑	–	[107]

a): *表示显著性, ↓表示减少, ↑表示增加, –表示没有数据

组装完整组分羧酶体, 进一步提高高等植物RuBisCO羧化效率.

总之, 近年来有关RuBisCO研究取得了显著进展, 未来利用转基因及基因编辑技术对RuBisCO大小亚基的功能位点进行深入研究, 寻找提高RuBisCO催化活性的关键位点, 并解析其复杂的分子生理机制, 将为在作物中构建高效RuBisCO, 提高作物光合效率进而提高粮食作物产量提供理论基础和新的研究思路.

致谢 感谢英国利物浦大学陈太钰博士在本文撰写过程中给予的指导.

参考文献

1 van Dijk M, Morley T, Rau M L, et al. A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. Nat Food, 2021, 2: 494–501

2 FAO, IFAD, UNICEF, et al. The State of Food Security and Nutrition in the World 2021. Transforming food systems for food security, improved nutrition and affordable healthy diets for all. Rome, FAO. 2021

3 Lin R C, Yang W Q, Wang B C, et al. Advances and perspectives in several areas of photosynthesis research (in Chinese). Sci Sin Vitae, 2021, 51: 1376–1384 [林荣呈, 杨文强, 王柏臣, 等. 光合作用研究若干前沿进展与展望. 中国科学: 生命科学, 2021, 51: 1376–1384]

4 Zhu G, Kurek I, Liu L. Chapter 20 engineering photosynthetic enzymes involved in CO_2-assimilation by gene shuffling. In: Rebeiz C A, Benning C, Bohnert H J, et al. The Chloroplast. Advances in Photosynthesis and Respiration. Dordrecht: Springer, 2010. 307–322

5 Bailey-Serres J, Parker J E, Ainsworth E A, et al. Genetic strategies for improving crop yields. Nature, 2019, 575: 109–118

6 Zhu X G, Long S P, Ort D R. Improving photosynthetic efficiency for greater yield. Annu Rev Plant Biol, 2010, 61: 235–261

7 Wei S, Li X, Lu Z, et al. A transcriptional regulator that boosts grain yields and shortens the growth duration of rice. Science, 2022, 377: eabi8455

8 Tcherkez G G B, Farquhar G D, Andrews T J. Despite slow catalysis and confused substrate specificity, all ribulose bisphosphate carboxylases may be nearly perfectly optimized. Proc Natl Acad Sci USA, 2006, 103: 7246–7251

9 Andersson I. Catalysis and regulation in Rubisco. J Exp Bot, 2007, 59: 1555–1568

10 Field C B, Behrenfeld M J, Randerson J T, et al. Primary production of the biosphere: integrating terrestrial and oceanic components. Science, 1998, 281: 237–240

11 Berg I A. Ecological aspects of the distribution of different autotrophic CO_2 fixation pathways. Appl Environ Microbiol, 2011, 77: 1925–1936

12 Bar-On Y M, Milo R. The global mass and average rate of rubisco. Proc Natl Acad Sci USA, 2019, 116: 4738–4743

13 Liu L N. Advances in the bacterial organelles for CO_2 fixation. Trends Microbiol, 2022, 30: 567–580

14 Kumar S. Structure and functional annotation of hypothetical proteins having putative Rubisco activase function from *Vitis vinifera*.

Bioinformation, 2015, 11: 11–16
15 Sui N, Huang F, Liu L N. Photosynthesis in phytoplankton: insights from the newly discovered biological inorganic carbon pumps. Mol Plant, 2020, 13: 949–951
16 Wildman S G, Bonner J. The proteins of green leaves; isolation, enzymatic properties and auxin content of spinach cytoplasmic proteins. Arch Biochem, 1947, 14: 381–413
17 Quayle J R, Fuller R C, Benson A A, et al. Enzymatic carboxylation of ribulose diphosphate. J Am Chem Soc, 1954, 76: 3610–3611
18 Wilson A T, Calvin M. The photosynthetic cycle. CO_2 dependent transients. J Am Chem Soc, 1955, 77: 5948–5957
19 Bowes G, Ogren W L, Hageman R H. Phosphoglycolate production catalyzed by ribulose diphosphate carboxylase. Biochem Biophys Res Commun, 1971, 45: 716–722
20 Somerville C R, Portis Jr. A R, Ogren W L. A mutant of *Arabidopsis thaliana* which lacks activation of RuBP carboxylase *in vivo*. Plant Physiol, 1982, 70: 381–387
21 Chapman M S, Suh S W, Cascio D, et al. Sliding-layer conformational change limited by the quaternary structure of plant RuBisCO. Nature, 1987, 329: 354–356
22 Hemmingsen S M, Woolford C, van der Vies S M, et al. Homologous plant and bacterial proteins chaperone oligomeric protein assembly. Nature, 1988, 333: 330–334
23 Hemmingsen S M, Ellis R J. Purification and properties of ribulosebisphosphate carboxylase large subunit binding protein. Plant Physiol, 1986, 80: 269–276
24 Lin M T, Occhialini A, Andralojc P J, et al. A faster Rubisco with potential to increase photosynthesis in crops. Nature, 2014, 513: 547–550
25 Aigner H, Wilson R H, Bracher A, et al. Plant RuBisCo assembly in *E. coli* with five chloroplast chaperones including BSD2. Science, 2017, 358: 1272–1278
26 Schneider G, Lindqvist Y, Branden C I. Rubisco: structure and mechanism. Annu Rev Biophys Biomol Struct, 1992, 21: 119–143
27 Schulz L, Guo Z, Zarzycki J, et al. Evolution of increased complexity and specificity at the dawn of form I Rubiscos. Science, 2022, 378: 155–160
28 Banda D M, Pereira J H, Liu A K, et al. Novel bacterial clade reveals origin of form I Rubisco. Nat Plants, 2020, 6: 1158–1166
29 West-Roberts J A, Matheus-Carnevali P B, Schoelmerich M C, et al. The Chloroflexi supergroup is metabolically diverse and representatives have novel genes for non-photosynthesis based CO_2 fixation. bioRxiv, 2021, doi: 10.1101/2021.08.23.457424
30 Badger M R, Sharwood R E. Rubisco, the imperfect winner: it's all about the base. J Exp Bot, 2023, 74: 562–580
31 Bracher A, Whitney S M, Hartl F U, et al. Biogenesis and metabolic maintenance of Rubisco. Annu Rev Plant Biol, 2017, 68: 29–60
32 Finn M W, Tabita F R. Modified pathway to synthesize ribulose 1,5-bisphosphate in methanogenic archaea. J Bacteriol, 2004, 186: 6360–6366
33 Tabita F R, McFadden B A. d-Ribulose 1,5-diphosphate carboxylase from *Rhodospirillum rubrum*. J Biol Chem, 1974, 249: 3459–3464
34 Tabita F R. Microbial ribulose 1,5-bisphosphate carboxylase/oxygenase: a different perspective. Photosynth Res, 1999, 60: 1–28
35 Liu D, Ramya R C S, Mueller-Cajar O. Surveying the expanding prokaryotic Rubisco multiverse. FEMS Microbiol Lett, 2017, 364
36 Tabita F R, Satagopan S, Hanson T E, et al. Distinct form I, II, III, and IV Rubisco proteins from the three kingdoms of life provide clues about Rubisco evolution and structure/function relationships. J Exp Bot, 2008, 59: 1515–1524
37 Sato T, Atomi H, Imanaka T. Archaeal type III RuBisCOs function in a pathway for AMP metabolism. Science, 2007, 315: 1003–1006
38 Frolov E N, Kublanov I V, Toshchakov S V, et al. Form III RubisCO-mediated transaldolase variant of the Calvin cycle in a chemolithoautotrophic bacterium. Proc Natl Acad Sci USA, 2019, 116: 18638–18646
39 Tabita F R, Hanson T E, Satagopan S, et al. Phylogenetic and evolutionary relationships of RubisCO and the RubisCO-like proteins and the functional lessons provided by diverse molecular forms. Phil Trans R Soc B, 2008, 363: 2629–2640
40 Hanson T E, Tabita F R. A ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO)-like protein from *Chlorobium tepidum* that is involved with sulfur metabolism and the response to oxidative stress. Proc Natl Acad Sci USA, 2001, 98: 4397–4402
41 Andersson I, Backlund A. Structure and function of Rubisco. Plant Physiol Biochem, 2008, 46: 275–291
42 Andrews T J. Catalysis by cyanobacterial ribulose-bisphosphate carboxylase large subunits in the complete absence of small subunits. J Biol Chem, 1988, 263: 12213–12219
43 Bracher A, Starling-Windhof A, Hartl F U, et al. Crystal structure of a chaperone-bound assembly intermediate of form I Rubisco. Nat Struct Mol Biol, 2011, 18: 875–880

44 Knight S, Andersson I, Brändén C I. Crystallographic analysis of ribulose 1,5-bisphosphate carboxylase from spinach at 2·4 Å resolution. J Mol Biol, 1990, 215: 113–160
45 Spreitzer R J. Role of the small subunit in ribulose-1,5-bisphosphate carboxylase/oxygenase. Arch Biochem Biophys, 2003, 414: 141–149
46 Jordan D B, Ogren W L. Species variation in the specificity of ribulose biphosphate carboxylase/oxygenase. Nature, 1981, 291: 513–515
47 Berry J O, Yerramsetty P, Zielinski A M, et al. Photosynthetic gene expression in higher plants. Photosynth Res, 2013, 117: 91–120
48 Berry J O, Mure C M, Yerramsetty P. Regulation of Rubisco gene expression in C_4 plants. Curr Opin Plant Biol, 2016, 31: 23–28
49 Bowman S M, Patel M, Yerramsetty P, et al. A novel RNA binding protein affects *rbcL* gene expression and is specific to bundle sheath chloroplasts in C_4 plants. BMC Plant Biol, 2013, 13: 138
50 Johnson X, Wostrikoff K, Finazzi G, et al. MRL1, a conserved pentatricopeptide repeat protein, is required for stabilization of *rbcL* mRNA in *Chlamydomonas* and *Arabidopsis*. Plant Cell, 2010, 22: 234–248
51 Houtz R L, Magnani R, Nayak N R, et al. Co- and post-translational modifications in Rubisco: unanswered questions. J Exp Bot, 2008, 59: 1635–1645
52 Gao X, Hong H, Li W C, et al. Downregulation of Rubisco activity by non-enzymatic acetylation of RbcL. Mol Plant, 2016, 9: 1018–1027
53 Khrebtukova I, Spreitzer R J. Elimination of the *Chlamydomonas* gene family that encodes the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase. Proc Natl Acad Sci USA, 1996, 93: 13689–13693
54 Wietrzynski W, Traverso E, Wollman F A, et al. The state of oligomerization of Rubisco controls the rate of synthesis of the Rubisco large subunit in *Chlamydomonas reinhardtii*. Plant Cell, 2021, 33: 1706–1727
55 Hinault M P, Ben-Zvi A, Goloubinoff P. Chaperones and proteases: cellular fold-controlling factors of proteins in neurodegenerative diseases and aging. J Mol Neurosci, 2006, 30: 249–266
56 Natalello A, Mattoo R U H, Priya S, et al. Biophysical characterization of two different stable misfolded monomeric polypeptides that are chaperone-amenable substrates. J Mol Biol, 2013, 425: 1158–1171
57 Hayer-Hartl M, Hartl F U. Chaperone machineries of Rubisco—the most abundant enzyme. Trends Biochem Sci, 2020, 45: 748–763
58 Georgopoulos C P, Hendrix R W, Casjens S R, et al. Host participation in bacteriophage lambda head assembly. J Mol Biol, 1973, 76: 45–60
59 Vitlin Gruber A, Feiz L. Rubisco assembly in the chloroplast. Front Mol Biosci, 2018, 5: 24
60 Bai C, Guo P, Zhao Q, et al. Protomer roles in chloroplast chaperonin assembly and function. Mol Plant, 2015, 8: 1478–1492
61 Liu C, Young A L, Starling-Windhof A, et al. Coupled chaperone action in folding and assembly of hexadecameric Rubisco. Nature, 2010, 463: 197–202
62 Feiz L, Williams-Carrier R, Wostrikoff K, et al. Ribulose-1,5-bis-phosphate carboxylase/oxygenase accumulation factor1 is required for holoenzyme assembly in maize. Plant Cell, 2012, 24: 3435–3446
63 Hauser T, Bhat J Y, Miličić G, et al. Structure and mechanism of the Rubisco-assembly chaperone Raf1. Nat Struct Mol Biol, 2015, 22: 720–728
64 Kolesinski P, Belusiak I, Czarnocki-Cieciura M, et al. Rubisco accumulation factor 1 from *Thermosynechococcus elongatus* participates in the final stages of ribulose-1,5-bisphosphate carboxylase/oxygenase assembly in *Escherichia coli* cells and *in vitro*. FEBS J, 2014, 281: 3920–3932
65 Fristedt R, Hu C, Wheatley N, et al. RAF2 is a RuBisCO assembly factor in *Arabidopsis thaliana*. Plant J, 2018, 94: 146–156
66 Wheatley N M, Sundberg C D, Gidaniyan S D, et al. Structure and identification of a pterin dehydratase-like protein as a ribulose-bisphosphate carboxylase/oxygenase (RuBisCO) assembly factor in the α-carboxysome. J Biol Chem, 2014, 289: 7973–7981
67 Feiz L, Williams-Carrier R, Belcher S, et al. A protein with an inactive pterin-4a-carbinolamine dehydratase domain is required for Rubisco biogenesis in plants. Plant J, 2014, 80: 862–869
68 Li C, Ding B, Ma X, et al. A temporal gradient of cytonuclear coordination of chaperonins and chaperones during RuBisCo biogenesis in allopolyploid plants. Proc Natl Acad Sci USA, 2022, 119: e2200106119
69 Xia L Y, Jiang Y L, Kong W W, et al. Molecular basis for the assembly of RuBisCO assisted by the chaperone Raf1. Nat Plants, 2020, 6: 708–717
70 Li Q, Jiang Y L, Xia L Y, et al. Structural insights into cyanobacterial RuBisCO assembly coordinated by two chaperones Raf1 and RbcX. Cell Discov, 2022, 8: 93
71 Sharwood R E, Ghannoum O, Kapralov M V, et al. Temperature responses of Rubisco from *Paniceae* grasses provide opportunities for improving C_3 photosynthesis. Nat Plants, 2016, 2: 16186
72 Carmo-Silva A E, Keys A J, Andralojc P J, et al. Rubisco activities, properties, and regulation in three different C_4 grasses under drought. J Exp

Bot, 2010, 61: 2355–2366
73 Galmés J, Kapralov M V, Copolovici L O, et al. Temperature responses of the Rubisco maximum carboxylase activity across domains of life: phylogenetic signals, trade-offs, and importance for carbon gain. Photosynth Res, 2015, 123: 183–201
74 Galmés J, Conesa M À, Díaz-Espejo A, et al. Rubisco catalytic properties optimized for present and future climatic conditions. Plant Sci, 2014, 226: 61–70
75 Prins A, Orr D J, Andralojc P J, et al. Rubisco catalytic properties of wild and domesticated relatives provide scope for improving wheat photosynthesis. J Exp Bot, 2016, 67: 1827–1838
76 Young J N, Heureux A M C, Sharwood R E, et al. Large variation in the Rubisco kinetics of diatoms reveals diversity among their carbon-concentrating mechanisms. J Exp Bot, 2016, 67: 3445–3456
77 Orr D, Alcântara A, Kapralov M V, et al. Surveying Rubisco diversity and temperature response to improve crop photosynthetic efficiency. Plant Physiol, 2016, 172: 717–3456
78 Hermida-Carrera C, Kapralov M V, Galmés J. Rubisco catalytic properties and temperature response in crops. Plant Physiol, 2016, 171: 2549–2561
79 Galmes J, Flexas J, Keys A J, et al. Rubisco specificity factor tends to be larger in plant species from drier habitats and in species with persistent leaves. Plant Cell Environ, 2005, 28: 571–579
80 Ghannoum O, Evans J R, Chow W S, et al. Faster Rubisco is the key to superior nitrogen-use efficiency in NADP-Malic enzyme relative to NAD-Malic enzyme C_4 grasses. Plant Physiol, 2005, 137: 638–650
81 Kubien D S, Whitney S M, Moore P V, et al. The biochemistry of Rubisco in *Flaveria*. J Exp Bot, 2008, 59: 1767–1777
82 Christin P A, Salamin N, Muasya A M, et al. Evolutionary switch and genetic convergence on *rbcL* following the evolution of C_4 photosynthesis. Mol Biol Evol, 2008, 25: 2361–2368
83 King W A, Gready J E, Andrews T J. Quantum chemical analysis of the enolization of ribulose bisphosphate: the first hurdle in the fixation of CO_2 by Rubisco. Biochemistry, 1998, 37: 15414–15422
84 Taylor T C, Andersson I. The structure of the complex between Rubisco and its natural substrate ribulose 1,5-bisphosphate. J Mol Biol, 1997, 265: 432–444
85 Newman J, Gutteridge S. The X-ray structure of Synechococcus ribulose-bisphosphate carboxylase/oxygenase-activated quaternary complex at 2.2-Å resolution. J Biol Chem, 1993, 268: 25876–25886
86 Taylor T C, Backlund A, Bjorhall K, et al. First crystal structure of Rubisco from a green alga, *Chlamydomonas reinhardtii*. J Biol Chem, 2001, 276: 48159–48164
87 Lin M T, Salihovic H, Clark F K, et al. Improving the efficiency of Rubisco by resurrecting its ancestors in the family Solanaceae. Sci Adv, 2022, 8: eabm6871
88 Matsumura H, Shiomi K, Yamamoto A, et al. Hybrid Rubisco with complete replacement of rice Rubisco small subunits by sorghum counterparts confers C_4 plant-like high catalytic activity. Mol Plant, 2020, 13: 1570–1581
89 Brand A, Tissier A. Control of resource allocation between primary and specialized metabolism in glandular trichomes. Curr Opin Plant Biol, 2022, 66: 102172
90 Laterre R, Pottier M, Remacle C, et al. Photosynthetic trichomes contain a specific Rubisco with a modified pH-dependent activity. Plant Physiol, 2017, 173: 2110–2120
91 Lin M T, Stone W D, Chaudhari V, et al. Small subunits can determine enzyme kinetics of tobacco Rubisco expressed in *Escherichia coli*. Nat Plants, 2020, 6: 1289–1299
92 Martin-Avila E, Lim Y L, Birch R, et al. Modifying plant photosynthesis and growth via simultaneous chloroplast transformation of Rubisco large and small subunits. Plant Cell, 2020, 32: 2898–2916
93 Spreitzer R J. Questions about the complexity of chloroplast ribulose-1,5-bisphosphate carboxylase/oxygenase. Photosynth Res, 1999, 60: 29–42
94 Parry M A J. Rubisco activity: effects of drought stress. Ann Bot, 2002, 89: 833–839
95 Hall N P, Keys A J. Temperature dependence of the enzymic carboxylation and oxygenation of ribulose 1,5-bisphosphate in relation to effects of temperature on photosynthesis. Plant Physiol, 1983, 72: 945–948
96 Brooks A, Farquhar G D. Effect of temperature on the CO_2/O_2 specificity of ribulose-1,5-bisphosphate carboxylase/oxygenase and the rate of

respiration in the light. Planta, 1985, 165: 397–406
97 Bernacchi C J, Singsaas E L, Pimentel C, et al. Improved temperature response functions for models of Rubisco-limited photosynthesis. Plant Cell Environ, 2001, 24: 253–259
98 Moore C E, Meacham-Hensold K, Lemonnier P, et al. The effect of increasing temperature on crop photosynthesis: from enzymes to ecosystems. J Exp Bot, 2021, 72: 2822–2844
99 Sage R F. Variation in the k_{cat} of Rubisco in C_3 and C_4 plants and some implications for photosynthetic performance at high and low temperature. J Exp Bot, 2002, 53: 609–620
100 Galmés J, Capó-Bauçà S, Niinemets Ü, et al. Potential improvement of photosynthetic CO_2 assimilation in crops by exploiting the natural variation in the temperature response of Rubisco catalytic traits. Curr Opin Plant Biol, 2019, 49: 60–67
101 Salesse-Smith C E, Sharwood R E, Busch F A, et al. Increased Rubisco content in maize mitigates chilling stress and speeds recovery. Plant Biotechnol J, 2020, 18: 1409–1420
102 Portis A R, Jr. A R. Rubisco activase—Rubisco's catalytic chaperone. Photosynth Res, 2003, 75: 11–27
103 Portis A R, Li C, Wang D, et al. Regulation of Rubisco activase and its interaction with Rubisco. J Exp Bot, 2008, 59: 1597–1604
104 Degen G E, Worrall D, Carmo-Silva E. An isoleucine residue acts as a thermal and regulatory switch in wheat Rubisco activase. Plant J, 2020, 103: 742–751
105 Fukayama H, Ueguchi C, Nishikawa K, et al. Overexpression of Rubisco activase decreases the photosynthetic CO_2 assimilation rate by reducing rubisco content in rice leaves. Plant Cell Physiol, 2012, 53: 976–986
106 Yamori W, Masumoto C, Fukayama H, et al. Rubisco activase is a key regulator of non-steady-state photosynthesis at any leaf temperature and, to a lesser extent, of steady-state photosynthesis at high temperature. Plant J, 2012, 71: 871–880
107 Suganami M, Suzuki Y, Kondo E, et al. Effects of overproduction of Rubisco activase on Rubisco content in transgenic rice grown at different N levels. Int J Mol Sci, 2020, 21: 1626
108 Suganami M, Suzuki Y, Tazoe Y, et al. Co-overproducing Rubisco and Rubisco activase enhances photosynthesis in the optimal temperature range in rice. Plant Physiol, 2020, 185: 108–119
109 Qu Y, Sakoda K, Fukayama H, et al. Overexpression of both Rubisco and Rubisco activase rescues rice photosynthesis and biomass under heat stress. Plant Cell Environ, 2021, 44: 2308–2320
110 Leakey A D B, Ainsworth E A, Bernacchi C J, et al. Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. J Exp Bot, 2009, 60: 2859–2876
111 Long S P, Ainsworth E A, Rogers A, et al. Rising atmospheric carbon dioxide: plants face the future. Annu Rev Plant Biol, 2004, 55: 591–628
112 Zhu X G, Portis A R, Long S P. Would transformation of C_3 crop plants with foreign Rubisco increase productivity? A computational analysis extrapolating from kinetic properties to canopy photosynthesis. Plant Cell Environ, 2004, 27: 155–165
113 Chen G Y, Yong Z H, Liao Y, et al. Photosynthetic acclimation in rice leaves to free-air CO_2 enrichment related to both ribulose-1,5-bisphosphate carboxylation limitation and ribulose-1,5-bisphosphate regeneration limitation. Plant Cell Physiol, 2005, 46: 1036–1045
114 Zhu C, Zhu J, Cao J, et al. Biochemical and molecular characteristics of leaf photosynthesis and relative seed yield of two contrasting rice cultivars in response to elevated [CO_2]. J Exp Bot, 2014, 65: 6049–6056
115 Wang W, He J, Wang Z, et al. Leaf characteristics of rice cultivars with a stronger yield response to projected increases in CO_2 concentration. Physiol Plant, 2021, 171: 416–423
116 Thompson M, Gamage D, Hirotsu N, et al. Effects of elevated carbon dioxide on photosynthesis and carbon partitioning: a perspective on root sugar sensing and hormonal crosstalk. Front Physiol, 2017, 8: 578
117 Li X, Kristiansen K, Rosenqvist E, et al. Elevated CO_2 modulates the effects of drought and heat stress on plant water relations and grain yield in wheat. J Agro Crop Sci, 2019, 205: 362–371
118 Hussain S, Ulhassan Z, Brestic M, et al. Photosynthesis research under climate change. Photosynth Res, 2021, 150: 5–19
119 Fluhr R, Kuhlemeier C, Nagy F, et al. Organ-specific and light-induced expression of plant genes. Science, 1986, 232: 1106–1112
120 Timko M P, Kausch A P, Castresana C, et al. Light regulation of plant gene expression by an upstream enhancer-like element. Nature, 1985, 318: 579–582
121 Yerramsetty P, Agar E M, Yim W C, et al. An *rbcL* mRNA-binding protein is associated with C_3 to C_4 evolution and light-induced production of Rubisco in *Flaveria*. J Exp Bot, 2017, 68: 4635–4649

122 Long S P, Taylor S H, Burgess S J, et al. Into the shadows and back into sunlight: photosynthesis in fluctuating light. Annu Rev Plant Biol, 2022, 73: 617–648

123 Laing W A, Christeller J T. A model for the kinetics of activation and catalysis of ribulose 1,5-bisphosphate carboxylase. Biochem J, 1976, 159: 563–570

124 Lorimer G H. The carboxylation and oxygenation of ribulose 1,5-bisphosphate: the primary events in photosynthesis and photorespiration. Annu Rev Plant Physiol, 1981, 32: 349–382

125 Lorimer G H, Badger M R, Andrews T J. The activation of ribulose-1,5-bisphosphate carboxylase by carbon dioxide and magnesium ions. Equilibria, kinetics, a suggested mechanism, and physiological implications. Biochemistry, 1976, 15: 529–536

126 Badger M R, Lorimer G H. Activation of ribulose-1,5-bisphosphate oxygenase. Arch Biochem Biophys, 1976, 175: 723–729

127 Perchorowicz J T, Raynes D A, Jensen R G. Light limitation of photosynthesis and activation of ribulose bisphosphate carboxylase in wheat seedlings. Proc Natl Acad Sci USA, 1981, 78: 2985–2989

128 Carmo-Silva A E, Salvucci M E. The regulatory properties of Rubisco activase differ among species and affect photosynthetic induction during light transitions. Plant Physiol, 2013, 161: 1645–1655

129 Lan Y, Woodrow I E, Mott K A. Light-dependent changes in ribulose bisphosphate carboxylase activase activity in leaves. Plant Physiol, 1992, 99: 304–309

130 Ruuska S A, Andrews T J, Badger M R, et al. The role of chloroplast electron transport and metabolites in modulating Rubisco activity in tobacco. Insights from transgenic plants with reduced amounts of cytochrome *b/f* complex or glyceraldehyde 3-phosphate dehydrogenase. Plant Physiol, 2000, 122: 491–504

131 Zhang N, Kallis R P, Ewy R G, et al. Light modulation of Rubisco in *Arabidopsis* requires a capacity for redox regulation of the larger Rubisco activase isoform. Proc Natl Acad Sci USA, 2002, 99: 3330–3334

132 Sage R F, Pearcy R W. The nitrogen use efficiency of C_3 and C_4 plants. Plant Physiol, 1987, 84: 959–963

133 Feller U, Anders I, Mae T. Rubiscolytics: fate of Rubisco after its enzymatic function in a cell is terminated. J Exp Bot, 2008, 59: 1615–1624

134 Yoneyama T, Tanno F, Tatsumi J, et al. Whole-plant dynamic system of nitrogen use for vegetative growth and grain filling in rice plants (*Oryza sativa* L.) as revealed through the production of 350 grains from a germinated seed over 150 days: a review and synthesis. Front Plant Sci, 2016, 7: 1151

135 Zhou B, Serret M D, Pie J B, et al. Relative contribution of nitrogen absorption, remobilization, and partitioning to the ear during grain filling in Chinese winter wheat. Front Plant Sci, 2018, 9: 1351

136 Zhou B, Serret M D, Elazab A, et al. Wheat ear carbon assimilation and nitrogen remobilization contribute significantly to grain yield. J Integr Plant Biol, 2016, 58: 914–926

137 Wada S, Hayashida Y, Izumi M, et al. Autophagy supports biomass production and nitrogen use efficiency at the vegetative stage in rice. Plant Physiol, 2015, 168: 60–73

138 Boussadia O, Steppe K, Zgallai H, et al. Effects of nitrogen deficiency on leaf photosynthesis, carbohydrate status and biomass production in two olive cultivars ‘Meski’ and ‘Koroneiki’. Sci Hortic, 2010, 123: 336–342

139 Yamori W, Nagai T, Makino A. The rate-limiting step for CO_2 assimilation at different temperatures is influenced by the leaf nitrogen content in several C_3 crop species. Plant Cell Environ, 2011, 34: 764–777

140 Gao J, Wang F, Hu H, et al. Improved leaf nitrogen reutilisation and Rubisco activation under short-term nitrogen-deficient conditions promotes photosynthesis in winter wheat (*Triticum aestivum* L.) at the seedling stage. Funct Plant Biol, 2018, 45: 840–853

141 Li Y, Yang X, Ren B, et al. Why nitrogen use efficiency decreases under high nitrogen supply in rice (*Oryza sativa* L.) seedlings. J Plant Growth Regul, 2011, 31: 47–52

142 Paul M J, Driscoll S P. Sugar repression of photosynthesis: the role of carbohydrates in signalling nitrogen deficiency through source:sink imbalance. Plant Cell Environ, 2010, 20: 110–116

143 Guiboileau A, Avila-Ospina L, Yoshimoto K, et al. Physiological and metabolic consequences of autophagy deficiency for the management of nitrogen and protein resources in *Arabidopsis* leaves depending on nitrate availability. New Phytol, 2013, 199: 683–694

144 Carmo-silva E, Scales J C, Madgwick P J, et al. Optimizing Rubisco and its regulation for greater resource use efficiency. Plant Cell Environ, 2015, 38: 1817–1832

145 Whitney S M, Houtz R L, Alonso H. Advancing our understanding and capacity to engineer nature’s CO_2-sequestering enzyme, Rubisco. Plant

Physiol, 2011, 155: 27–35
146 Iqbal W A, Miller I G, Moore R L, et al. Rubisco substitutions predicted to enhance crop performance through carbon uptake modelling. J Exp Bot, 2021, 72: 6066–6075
147 Kanevski I, Maliga P, Rhoades D F, et al. Plastome engineering of ribulose-1,5-bisphosphate carboxylase/oxygenase in tobacco to form a sunflower large subunit and tobacco small subunit hybrid. Plant Physiol, 1999, 119: 133–142
148 Whitney S M, Andrews T J. Plastome-encoded bacterial ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) supports photosynthesis and growth in tobacco. Proc Natl Acad Sci USA, 2001, 98: 14738–14743
149 Whitney S M, Sharwood R E, Orr D, et al. Isoleucine 309 acts as a C_4 catalytic switch that increases ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) carboxylation rate in *Flaveria*. Proc Natl Acad Sci USA, 2011, 108: 14688–14693
150 Sharwood R E, von Caemmerer S, Maliga P, et al. The catalytic properties of hybrid Rubisco comprising tobacco small and sunflower large subunits mirror the kinetically equivalent source Rubiscos and can support tobacco growth. Plant Physiol, 2008, 146: 83–96
151 Whitney S M, Birch R, Kelso C, et al. Improving recombinant Rubisco biogenesis, plant photosynthesis and growth by coexpressing its ancillary RAF1 chaperone. Proc Natl Acad Sci USA, 2015, 112: 3564–3569
152 Salesse-Smith C E, Sharwood R E, Busch F A, et al. Overexpression of Rubisco subunits with RAF1 increases Rubisco content in maize. Nat Plants, 2018, 4: 802–810
153 Gunn L H, Martin Avila E, Birch R, et al. The dependency of red Rubisco on its cognate activase for enhancing plant photosynthesis and growth. Proc Natl Acad Sci USA, 2020, 117: 25890–25896
154 Chen T, Riaz S, Davey P, et al. Producing fast and active Rubisco in tobacco to enhance photosynthesis. Plant Cell, 2023, 35: 795–807
155 Lin M T, Occhialini A, Andralojc P J, et al. β-Carboxysomal proteins assemble into highly organized structures in *Nicotiana* chloroplasts. Plant J, 2014, 79: 1–12
156 Long B M, Hee W Y, Sharwood R E, et al. Carboxysome encapsulation of the CO_2-fixing enzyme Rubisco in tobacco chloroplasts. Nat Commun, 2018, 9: 3570
157 Lin M T, Orr D J, Worrall D, et al. A procedure to introduce point mutations into the Rubisco large subunit gene in wild-type plants. Plant J, 2021, 106: 876–887
158 Khumsupan P, Kozlowska M A, Orr D J, et al. Generating and characterizing single- and multigene mutants of the Rubisco small subunit family in *Arabidopsis*. J Exp Bot, 2020, 71: 5963–5975
159 Pearce FG , Brunke J E. Is now the time for a Rubiscuit or Ruburger? Increased interest in Rubisco as a food protein. J Exp Bot, 2023, 74: 627–637
160 Gao C. Genome engineering for crop improvement and future agriculture. Cell, 2021, 184: 1621–1635
161 Huang L, Li Q, Zhang C, et al. Creating novel *Wx* alleles with fine-tuned amylose levels and improved grain quality in rice by promoter editing using CRISPR/Cas9 system. Plant Biotechnol J, 2020, 18: 2164–2166
162 Xu Y, Lin Q, Li X, et al. Fine-tuning the amylose content of rice by precise base editing of the *Wx* gene. Plant Biotechnol J, 2021, 19: 11–13
163 Gao H, Gadlage M J, Lafitte H R, et al. Superior field performance of waxy corn engineered using CRISPR-Cas9. Nat Biotechnol, 2020, 38: 579–581
164 Zeng D, Liu T, Ma X, et al. Quantitative regulation of *Waxy* expression by CRISPR/Cas9-based promoter and 5′UTR-intron editing improves grain quality in rice. Plant Biotechnol J, 2020, 18: 2385–2387
165 Kang B C, Bae S J, Lee S, et al. Chloroplast and mitochondrial DNA editing in plants. Nat Plants, 2021, 7: 899–905
166 Li R, Char S N, Liu B, et al. High-efficiency plastome base editing in rice with TAL cytosine deaminase. Mol Plant, 2021, 14: 1412–1414
167 Iqbal W A, Lisitsa A, Kapralov M V. Predicting plant Rubisco kinetics from RbcL sequence data using machine learning. J Exp Bot, 2023, 74: 638–650
168 Sharwood R E, Quick W P, Sargent D, et al. Mining for allelic gold: finding genetic variation in photosynthetic traits in crops and wild relatives. J Exp Bot, 2022, 73: 3085–3108
169 Yoon D K, Ishiyama K, Suganami M, et al. Transgenic rice overproducing Rubisco exhibits increased yields with improved nitrogen-use efficiency in an experimental paddy field. Nat Food, 2020, 1: 134–139
170 Kanno K, Suzuki Y, Makino A. A small decrease in rubisco content by individual suppression of *RBCS* genes leads to improvement of photosynthesis and greater biomass production in rice under conditions of elevated CO_2. Plant Cell Physiol, 2017, 58: 635–642

171 Fukayama H, Mizumoto A, Ueguchi C, et al. Expression level of Rubisco activase negatively correlates with Rubisco content in transgenic rice. Photosynth Res, 2018, 137: 465–474

172 Suzuki Y, Fujimori T, Kanno K, et al. Metabolome analysis of photosynthesis and the related primary metabolites in the leaves of transgenic rice plants with increased or decreased Rubisco content. Plant Cell Environ, 2012, 35: 1369–1379

173 Suzuki Y, Makino A. Availability of Rubisco small subunit up-regulates the transcript levels of large subunit for stoichiometric assembly of its holoenzyme in rice. Plant Physiol, 2012, 160: 533–540

174 Ishikawa C, Hatanaka T, Misoo S, et al. Functional incorporation of sorghum small subunit increases the catalytic turnover rate of Rubisco in transgenic rice. Plant Physiol, 2011, 156: 1603–1611

Research progress in plant RuBisCO

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Improving photosynthesis is one of the promising ways to increase crop yields in the future. Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), a key enzyme in the carbon assimilation process, catalyzes the carboxylation of CO_2 with RuBP, thus converts inorganic carbon into organic carbon. RuBisCO has a slow catalytic rate and poor capability in discriminating between the competing substrates, CO_2 and O_2, therefore it is called "low-efficiency enzyme". Given the importance of RuBisCO in the photosynthetic carbon assimilation process and its potential for future applications in improving crop photosynthetic efficiency and yield, the genetic modification of RuBisCO has become a hotspot and frontier in the field of photosynthesis research. This review systematically summarizes the classification of RuBisCO, its folding and assembly mechanism, natural variation, and response mechanism to the natural environment. It mainly focuses on the research progress of RuBisCO genetic engineering, and also discusses future perspectives in order to provide a guidance for future research.

RuBisCO, RuBisCO activase, natural variation, environmental response, RuBisCO genetic engineering

doi: 10.1360/SSV-2022-0275