核酮糖-1,5-二磷酸羧化酶/加氧酶活化酶在植物抗逆性中的作用

陈候鸣, 陈跃, 王盾, 蒋德安*

浙江大学生命科学学院, 植物生理与植物生物化学国家重点实验室, 杭州310058

摘要: 自上世纪80年代核酮糖-1,5-二磷酸羧化酶/加氧酶活化酶(RuBisCO activase, RCA)被发现后, 一直广受关注, 其酶学特性、体内外活化及其对光合作用的影响、体内外活性调节机制、基因和分子结构、反义突变和超表达等研究, 已成为了光合研究领域的一大热点。本文结合国内外研究进展, 就RCA分子结构、与光合作用的关系, 特别是其在植物抗逆中的作用等方面进行综述。

关键词: 核酮糖-1,5-二磷酸羧化酶/加氧酶活化酶; 光合作用; 非生物逆境

1982年, Somerville等研究者发现了只能在很高的CO_2浓度下生存的拟南芥(*Arabidopsis thaliana*)突变体, 后经证实其主要原因是体内核酮糖-1,5-二磷酸羧化酶/加氧酶(ribulose-1,5-bisphosphate carboxylase/oxygenase, RuBisCO)没能有效活化(Somerville等1982; Salvucci等1985, 1987)。蛋白表达谱和mRNA分析表明, 该突变体叶绿体中缺少41和45 kDa的两条可溶性多肽, 因而该蛋白被定名为RuBisCO活化酶(RuBisCO activase, RCA) (Portis 1995)。国内外对RCA的酶学特性和体内外活化RuBisCO的作用(唐如航等1997; Robinson等1988; Robinson和Portis 1989a, b; Salvucci等1987; Wang等1992, 1993)、RCA自身的活性调节机制(Shen等1991; Van de Loo和Salvucci 1998; Zhang等2002; Zhang和Portis 1999)及其在植物光合作用中的重要性等均有报道(蒋德安等2001; 翁晓燕等2002; 姜振升等2010; He等1997; Jiang等2001; Jin等2008; Martinez等1997; Morales等1999; Wang等2009; Yin等2014)。人们对*RCA*体内外表达动态或调节(唐如航和贾军伟1997; 张峰等2002; Orozco和Ogren 1993; Qu等2011; Wang等2003; Watillon等1993)、基因结构组成也有较深入的认识(Li等1993, 1999; To等1999; Werneke等1988a, 1988b, 1989; Zhang和Komatsu 2000), *RCA*反义突变和超表达等研究进一步证实了RCA的生理功能(Hammond等1998; Jiang等1994; Jin等2004, 2006; Kurek等2007; Mate和Andrews 1993; Shen和Ogren 1992; Wang等2010; Yang等2012), 这方面国内外曾有不少综述(韩鹰等2000; 李海霞等2010; 唐如航和李立人1998; 翁晓燕等1998; 张国等2004; Parry等2003; Portis 1995, 2003; Portis和Salvucci 2002; Portis等2008)。本文在本实验室研究基础上, 结合国内外近年研究进展, 就RCA分子结构及其与植物光合作用的关系、在抗逆性中的作用和结合蛋白等方面进行综述。

1 RCA分子基础和蛋白结构

1.1 RCA分子基础

RCA是一种由核基因编码的可溶性叶绿体蛋白, 普遍存在于高等植物、绿藻和蓝细菌中(Werneke等1988a, b; Mueller-Cajar等2011, 2014)。不同植物中*RCA*基因虽有差异, 但基因表达出的RCA蛋白则主要为41~43 kDa的小同工型(RCAII或RCA_S)和45~46 kDa的大同工型(RCAI或RCA_L)两种(Portis 2003), 或β和α型。在菠菜(*Spinacia oleracea*) (Werneke等1988b)、拟南芥(Werneke等1989)、水稻(*Oryza sativa*) (Chen等2014; To等1999)和小麦(*Triticum aestivum*) (Law和Crafts-Brandner 2001)中, 仅有一个*RCA*基因编码, 但在大麦(*Hordeum vulgare*)和棉花(*Gossypium hirsutum*)中有两个*RCA*基因编码; 大麦中一个基因可产生两个同工型(isoform), 另一个只产生RCAII (Rundle和Zielinski 1991; Salvucci等2003)。尽管在烟草(*Nicotiana tabacum*) (Qian和Rodermel 1993)和大豆(*Glycine max*) (Yin等2010)中有多个*RCA*基因编码, 但只能产生RCAII蛋白。两个同工型的形成主要是*RCA*前体mRNA的选择性剪接结果, 如水稻中前体mRNA有3个内含子和4个外显子, 选择性剪接的一种是剪除3个内含子, 保留全部的1~4外显子, 这种剪接导致外显子2的

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* 通讯作者(E-mail: dajiang@zju.edu.cn)。

位点上有终止信号UGA, 从而使外显子2大部分和外显子3、4均不能表达出多肽序列, 形成的蛋白分子质量为41 kDa的RCA_S; 另一种剪接方式是剪去3个内含子及2、3两个外显子, 使1、4外显子直接拼接, 翻译出的蛋白羧基端多38个氨基酸, 为45 kDa的RCA_L (Chen等2015; Wang等2009, 2010; To等1999)。尽管*RCA*选择性剪接发生在绝大部分植物中, 但人们对植物中这种选择性剪接的机制还不清楚(Reddy 2007)。随着模式植物基因组测序工作的完成, 生物信息学分析的结果表明选择性剪接现象在开花植物中普遍存在。Wang和Brendel (2006)研究表明, 拟南芥有4 707个基因(占总基因的22%), 水稻有6 568个基因(占总基因的21.2%)都要经历选择性剪接(Reddy 2007)。选择性剪接通过改变mRNA前体的剪接位点产生两个或两个以上mRNA产物, 可以产生多种不同的同工型蛋白, 而这些新增的同工型可能缺失或新增某些生物学功能, 或者具有不同的细胞定位, 亦或具有不同的酶活性和蛋白稳定性。RCA的两个同工型虽均具有活化RuBisCO的功能, 但表现为最大催化能力和动力学的不同(Shen等1991), 拟南芥光调节的RuBisCO活化需要通过硫氧还蛋白*f*参与的RCA_L的调节(Zhang和Komatsu等2000; Zhang和Portis 1999; Zhang等2002)。已知与RCA_S相比, RCA_L延长的羧基端含有氧化还原敏感的半胱氨酸残基(Portis 2003)。通过分析水稻常温和高温下RCA同工型的变化及不同同工型的超表达水稻的光合作用及其体内RuBisCO初始活力和总活力关系, 我们认为水稻中大小同工型主要功能不同, 前者主要与逆境诱导有关, 后者主要与RuBisCO的活化和光合速率有关(Chen等2015; Wang等2010)。

1.2 RCA蛋白空间结构

现已明确RCA是与多种细胞活性相关的ATP酶蛋白家族(AAA^+)成员, AAA^+蛋白的普遍特点是具有一个或多个AAA^+基序拷贝, 具有环状结构。RCA蛋白具有Walker A、Walker B、Sensor 1、Box VII和Sensor 2结构域, 有保守的核苷酸结合位点, ATP和Mg^{2+}能够催化其形成低聚体(Robinson等1988; Robinson和Portis 1989a, b; Neuwald等1999)。实验表明RCA以聚合体的形式催化RuBisCO活化(Portis 2003; Portis等2008; Wang等1993)。随着近年结构生物学研究的深入, 通过晶体衍射和冷冻电镜技术已解析了拟南芥(Hasse等2015)、烟草(Stotz等2011)及球形红假单胞菌(Mueller-Cajar等2011, 2014)的RCA立体结构。拟南芥和烟草RCA的AAA^+结构域具有这个蛋白家族的典型结构, 中心的β折叠链接着α螺旋和圆环, C端的小结构域由多个α螺旋缠绕而成; 除去信号肽部分, 他们在序列上有84%的相似性; 二者在形成低聚体时, 空间结构差异较大, 六聚体AtRCA的空间长度明显长于NtRCA, 而内在蛋白孔径AtRCA却明显小于NtRCA (图1-A和B; Hasse等2015)。这正解释了为什么以拟南芥为代表的RCA不能有效地催化以烟草为代表的RuBisCO, 反之亦然。

目前确认RCA以六聚体的形式行使活化RuBisCO的功能(Henderson等2013b, Mueller-Cajar等2011, 2014)。Henderson等(2013b)通过模型提出了假说, 认为RCA先由单体聚合成二聚体, 再由3个二聚体聚合成为六聚体环, 二聚体和六聚体可以各自形成更大的多聚体(图1-C), 但大于六聚体的多聚体其功能还不清楚。然而, RCA六聚体催化功能的模型全是建立在RCA_S同源六聚体环模型上, RCA_S是否与RCA_L形成多聚体, 或RCA_L能否形成多聚体还不清楚。Chen等(2015)利用生物信息学分析表明, 水稻RCA大小同工型的立体结构基本一致, 两个同工型的AAA^+区域是保守的。如图2所示, RCA_L与RCA_S的结构差异主要来自RCA_L C端38个氨基酸(图2-C, b), 这导致RCA_L比RCA_S多一开口(图2-C, d)。此外, 在C端附近还存在少量结构差异, 这种差异可能导致两个同工型各自有自己不同的结合蛋白, 从而引起同工型间功能的差异(Chen等2015)。

1.3 RCA与RuBisCO活化和光合速率的关系

RuBisCO催化卡尔文–本森循环(Calvin–Benson cycle)中核酮糖-1,5-二磷酸(ribulose-1,5-bisphosphate, RuBP)与CO_2形成两分子3-磷酸甘油酸(3-phosphoglyceric acid, 3-PGA)或光呼吸中RuBP与O_2结合形成1分子磷酸乙醇酸和1分子3-PGA的反应, 该酶处于光合碳还原或碳氧化两个相反但又相互关联的循环交叉点上, 是决定净光合速率最重要的因素。公认的RuBisCO催化活性需要第201位的赖氨酸与CO_2结合形成氨基甲酰化的赖氨酸残基, 再与

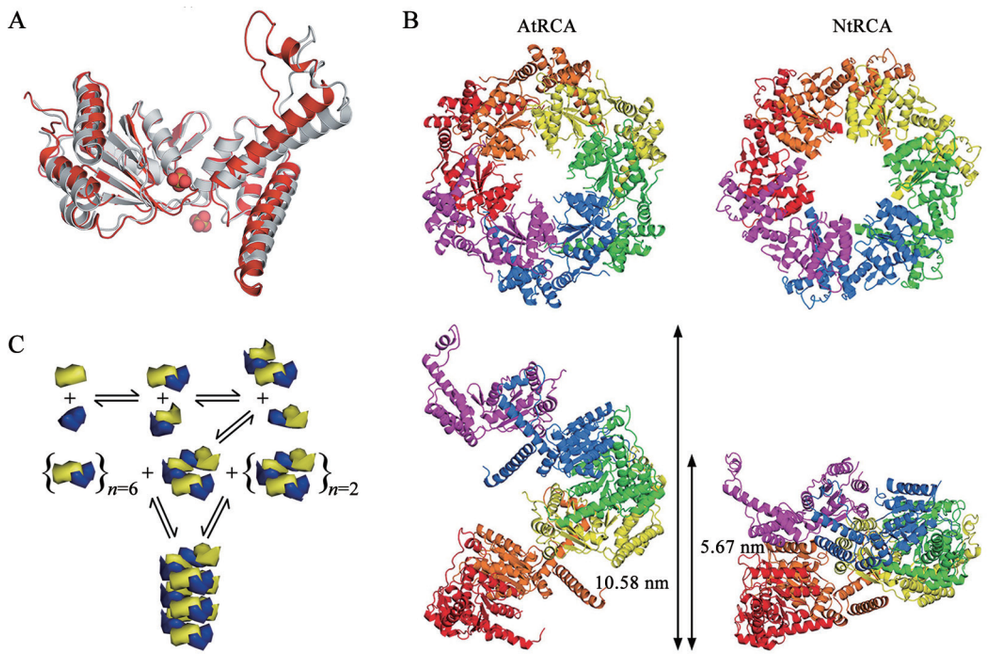

图1 RCA蛋白三维结构和多聚体形成模式

Fig.1 Three-dimensional structure and model of multipolymer formation of RCA

A: RCA单体结构, 红色为拟南芥AtRCA, 灰色为烟草NtRCA; B: 拟南芥和烟草RCA的六聚体结构; C: RCA多聚体的可能形成方式。A和B参考自Hasse等(2015), C参考自Henderson等(2013b)并略有改动。

Mg^{2+}形成三元复合物(enzyme-CO_2-Mg^{2+}, ECM), 才能进行羧化和加氧反应。但在这个ECM的活化态形成前, 处于开放状态的RuBisCO在环6和C端之间存在一个开放区域, 这个区域易和极微量的RuBP结合, 形成载脂RuBisCO (enzyme-RuBP, ER), 抑制ECM的形成。此外, 在不少植物中, 其ECM也易和2-羧基-D-阿拉伯糖醇-1-磷酸(2-carboxy-D-arabinitol 1-phosphate, CA1P), 木酮糖-1,5-二磷酸(D-xylulose 1,5-bisphosphate, XuBP)和D-甘油基-2,3-戊二酮糖-1,5-二磷酸(D-glycero-2,3-pentodiulose 1,5- bisphosphate, PDBP)等磷酸糖抑制物结合, 抑制其催化能力(Portis 2003; Portis等2008; Robinson和Portis 1989a, b; Salvucci和Ogren 1996; Spreiter和Salvucci 2002)。

RCA对于RuBisCO的活化就是通过解离ER或去除RuBisCO催化位点上的抑制物, 使CO_2更快地与RuBisCO 201位的赖氨酸发生氨甲酰化作用, 并与Mg^{2+}形成具有活性的三元复合物——ECM。RCA对RuBisCO活化过程可能涉及RCA对RuBisCO上特殊位点的识别、RCA和RuBisCO空间构象的变化、RCA-RuBisCO复合体的形成和分离等步骤(Portis 2003; Portis和Salvucci 2002; Portis等2008)。早期发现RCA和RuBisCO大亚基之间可以发生化学交联(Yokota和Tsujimoto 1992)和免疫共沉淀(Sánchez de Jiménez等1995; Zhang和Komatsu等2000), 之后通过直接突变氨基酸位点和叶绿体转化等手段产生突变RuBisCO和RCA以研究两者结合位点, 证实RuBisCO大亚基89~94位氨基酸区域和RCA 311~314位氨基酸区域(依据菠菜序列)是其结合位点, 而RuBisCO小亚基并不在RuBisCO和RCA结合过程中起作用(Larson等1997; Duff等2000; Ott等2000; Li等2005; Portis等2008; Wachter等2013; Mueller-Cajar等2011, 2014)。RCA-RuBisCO复合物使RuBisCO发生构象改变, 最终释放抑制物形成活化状态。

Martinez等(1997)报告玉米(*Zea mays*)籽粒灌浆期, RCA与RuBisCO活性和光合作用的联系, 表明了RCA在调节光合作用中的重要性。本实验室对水稻叶片衰老(翁晓燕等2002; 蒋德安等2001)、光合作用日变化(Jiang等2001; Wang等2003)和水

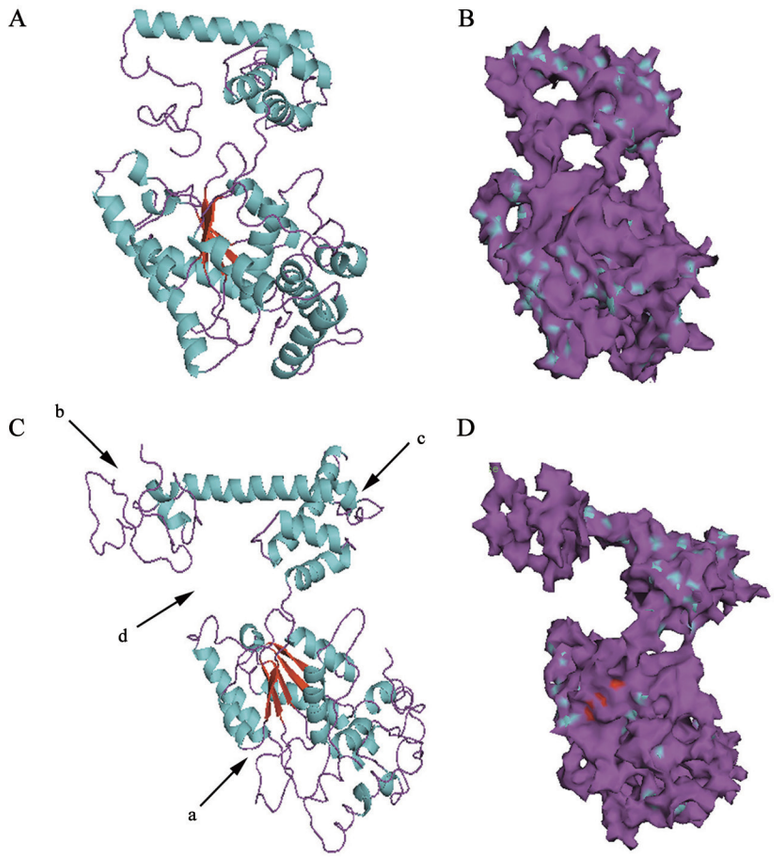

图2 RCA_L和RCA_S三维结构的差异

Fig.2 Difference in three-dimensional structures between RCA_L and RCA_S

A和B、C和D是分别基于Protein Data Bank数据库的RCA_L和RCA_S三维结构预测; a、b、c和d分别表示RCA_L和RCA_S三维结构的不同点。参考自Chen等(2015)并略有改动。

稻不同品种(翁晓燕等2002)的研究表明, 同一条件下RCA含量与光合作用密切相关, 表现为高RCA含量的材料, 有高的光合速率, 衰老时RCA含量下降慢, 光合下降也迟。亚硫酸氢钠提高光合作用是因其促进环式光合磷酸化的同时, 提高了RuBisCO活性(Yang等2008), 这与RCA的表达增加一致(Chen等2014)。*RCA*反义突变降低其RCA含量的同时, 往往伴随光合速率的下降(Hammond等1998; Jin等2004, 2006; Mate和Andrews 1993), 超表达*RCA*能一定程度上提高水稻光合速率(Wang等2010)。123个富有遗传多样性的玉米杂交系产量与*RCA*基因的表达呈正相关, 且与其他C_4基因的表达相关(Yin等2014)。但也有报道在烟草、拟南芥和水稻的*RCA*反义RNA植株中伴随RCA含量的下降存在RuBisCO积累的现象(Mate和Andrews 1993; Eckardt等1997; Jin等2006)。Fukayama等(2009, 2012)发现在长期高浓度CO_2环境中培养的水稻出现*RCA*基因表达上调而RuBisCO小亚基基因家族表达下调的现象, 后又在水稻中过表达大麦*RcaA*和玉米*Rca*基因, 发现在转基因水稻叶中存在因RuBisCO含量下调而导致的光合CO_2同化效率降低的现象, 同时排除了RuBisCO大小亚基转录水平的影响, 提出可能存在RCA对RuBisCO的转录后调控。

2 RCA在植物抗逆性中的作用

2.1 RCA与温度胁迫

Sage等(2008)认为RCA或能在植物适应日益变暖的全球气候下发挥重要作用。高温处理表明菠菜、棉花和小麦的RuBisCO失活分别与RCA失活有关(Crafts-Brandner 1997; Crafts-Brandner和

Salvucci 2000; Law和Crafts-Brandner 2001)。高温降低光合碳同化首先是影响体内RuBisCO的活化, Eckardt和Portis (1997)利用菠菜RuBisCO进行体外耐热活性实验发现超过60°C, RuBisCO活力就会下降约一半, 但在体内, 当温度高于40°C时就会出现RuBisCO活力的显著下降(Eckardt和Portis 1997; Feller等1998; Law和Craft-Brandner 1999, 2001), 这说明高温对RuBisCO的抑制作用很可能不是直接作用于RuBisCO本身, 而是可能通过抑制RCA的活性来实现的。Sánchez de Jiménez等(1995)发现, 玉米遭遇高温胁迫时会产生一条新的RCA多肽, 该多肽随温度恢复而消失, 研究发现在小麦和棉花中都存在相同现象, 推测高温诱导RCA的形成可能普遍存在于植物中(Law和Crafts-Brandner 2001; Law等2001)。不少研究表明, RCA在减轻高温对RuBisCO的钝化或活性的恢复从而维持植物在高温下一定的光合速率中发挥关键作用(Crafts-Brandner 1997; Crafts-Brandner和Salvucci 2000; Law和Crafts-Brandner 2001; Salvucci和Crafts-Brandner 2004a, b; Salvucci等2001, 2006)。Kurek等(2007)在拟南芥中把热稳定性各异的*RCAI*突变体引入RCA缺失型的拟南芥株系中, 这种转基因植物在每天经受4 h、30°C热胁迫下, 具备比野生型株系发育好、光合速率高和种子产量高的特点。最近Scafaro等(2016)报告不同耐热性水稻与RCA的关系, 发现野生稻RCA热稳定性高的材料, 在45°C高温下叶片伸展和光合几乎没有影响, 而RCA热稳定性低的栽培稻, 高温下光合速率降了50%多, 再次强调了RCA在提高植物耐热性中的重要性。通过分别超表达RCA不同同工型, 证明在常温下超表达RCA_S水稻比超表达RCA_L和对照野生型有更高的光合速率, 特别是由暗到光转化过程中光合速率上升加快, 达到稳定时间缩短; 而在高温下, 超表达RCA_L的株系比超表达RCA_S和对照野生型能维持较高的光合速率, 首次提出RCA的大小同工型具有不同的主要生理功能(Wang等2010)。

近年来, 有关RCA对低温胁迫响应研究也有报告, Jurczyk等(2016)研究发现黑麦草(*Lolium perenne*)在低温条件下通过增强RCA表达, 特别是增强可变剪接RCA_L的表达, 从而提升RuBisCO活力来维持光合碳同化速率以抵抗胁迫。姜振升等(2010)研究发现在低温弱光条件下黄瓜(*Cucumis sativus*)幼苗RuBisCO和RCA均出现表达下调的现象, 发现二者对低温弱光的响应和净光合速率(P_n)基本一致, 他们认为低温弱光下黄瓜幼苗P_n下降可能是由于RuBisCO和RCA表达下调所致。

2.2 RCA和其他非生物胁迫

RCA与其他非生物胁迫的关系也有报道, 树木在臭氧胁迫下, RuBisCO、RCA蛋白和mRNA表达下降(Pelloux等2001; Dizengremel 2001)。水稻干旱处理实验中, 耐旱品种RCA等蛋白表达显著上调(Salekdeh等2002)。近年来蛋白组学研究表明RCA在34种植物中参与对盐胁迫的响应(Zhang等2012), 它还参与桑科植物(Guha等2013)、水稻(Ji等2012; Salekdeh等2002)和大麦(Rollins等2013)对干旱以及马铃薯(*Solanum tuberosum*)中重金属胁迫的响应(Son等2014)。此外, Komatsu等(1996)报道了水稻中的赤霉素结合蛋白是与RCA同源的, 且与赤霉素信号传递过程中的Ca^{2+}依赖蛋白激酶有关(Sharma和Komatsu 2002)。RCA还参与植物叶片衰老的调节(He等1997; Shan等2011)。Orozco和Ogren (1993)将菠菜RCA基因启动子转化到烟草中, 发现其含有多个光诱导和组织特异元件。Qu等(2011)发现马铃薯RCA启动子上光诱导和组织特异性元件主要分布在启动子上游约220 bp范围内。Yang等(2012)通过转化该水稻启动子区域到拟南芥中, 确认水稻启动子区域也含有多种光诱导元件, 同时确定了一些和核蛋白互作的区域。Chen等(2015)利用生物信息学手段分析水稻RCA基因启动子上游200 bp区域, 发现除光诱导元件外, 还存在多种与非生物胁迫响应相关的顺式作用元件; 通过分析不同物种RCA蛋白结构和进化关系, 发现一级结构中RCA在不同物种间具有保守区域, 差异主要体现在二级结构、结合位点和三维结构三方面。研究证明在热、冷、盐和渗透胁迫条件下水稻叶片RCA的转录和翻译水平都明显升高, 并且RCA_L上升更多。逆境诱导叶绿体类囊体中RCA_L的数量显著积累, 叶绿体基质的RuBisCO-RCA复合体中RCA_L数量迅速增加(Chen等2015)。

3 RCA与分子伴侣蛋白的关系

3.1 分子伴侣

分子伴侣是一类与其他蛋白互作, 帮助其他

蛋白正确折叠和组装形成有功能的蛋白且其自身不是功能或结构组成的蛋白。研究已揭示这类伴侣蛋白(chaperone, Cpn)的结构, 发现它们参与新合成的蛋白结构的正确折叠, 在修复逆境引起的结构损坏和蛋白质内稳态等方面有重要作用(Balch等2008; Chiti和Dobson 2006; Henderson和Martin 2011; Henderson等2013a; Kim等2013b; Morimoto 2008; Tuccinardi 2011)。Cpn虽广泛存在于植物中, 但对其的功能研究仅有一些零星的报道, 如涉及质体发育(Apuya 2001; Jiang等2014; Suzuki等2009; Peng等2011; Kim等2013b)、质体辅酶I (II)脱氢酶复合体(Ndh) H亚基的装配以及叶绿体RuBisCO大亚基(rbcL)的折叠(Brocchieri和Karlin 2000; Kim等2013a)。

已发现的Cpn有两类: 一类是在细菌、叶绿体以及线粒体中发现的GroE类型, 另一类是在古菌和真核细胞质中发现的TCP1型(Brocchieri和Karlin 2000; Horwich等2009; Kim等2013b)。最初人们从大肠杆菌的基因产物GroEL中认识到这类对蛋白组装和细胞发挥功能有重要意义的蛋白(Georgopoulos等1972), 开始了对这类蛋白结构和功能的广泛研究。早期Cpn60作为植物中叶绿体RuBisCO大亚基的结合蛋白, 参与RuBisCO全酶的组装而受到人们的关注(Hemmingsen和Ellis 1986), 后来表明这两类蛋白是同系的, 代表一类新的分子伴侣——Cpn60。高等植物的线粒体和叶绿体中都存在核编码的Cpn60, 它们和细菌中的Cpn60在蛋白氨基酸序列的同源性上高度保守(Brocchieri和Karlin 2000; Horwich等2009; Kim等2013b; Sakamoto和Ohkuma 2010)。

电子显微镜和晶体衍射研究表明, 与细菌和线粒体含有的GroEL蛋白是由同源14聚体组成不同, 叶绿体中的Cpn60由异源14聚体(α7β7)组成(Levy-Rimler等2002)。Dickson等(2000)通过体外重组实验获得了由β构成的14聚体(β14)和由α和β共同构成的14聚体(α/β14), 在电子显微镜下观察, 发现两者结构无明显区别, 且它们都能帮助蛋白质折叠, 但是与它们特异结合的Cpn10却完全不一样, α/β14在功能上能与来自细菌、线粒体和叶绿体中的Cpn10相互作用, 而β14在参与蛋白折叠过程时, 只与线粒体Cpn10相互作用。

拟南芥基因组中已预测有7个基因可能编码质体Cpn60, 包括2个Cpn60α、4个Cpn60β和1个类似于Cpn60β的假基因。Cpn60α1、Cpn60β1和Cpn60β2是主要表达蛋白, Cpn60α2、Cpn60β3和Cpn60β4表达水平比较低(Hill和Hemmingsen 2001; Peltier等2006; Weiss等2009)。拟南芥中Cpn60α1敲除后能够引起质体发育缺陷, 从而导致胚胎死亡(Apuya等2001)。Cpn60α和Cpn60β是拟南芥幼苗发育过程中叶绿体分裂所需要的, 单突变叶片发黄, 双突变体成白化苗致死(Suzuki等2009)。此外, 极低的Cpn60β4蛋白可参与拟南芥NdhH亚基的装配(Peng等2011)。反义Cpn60β转化的烟草表现出生长缓慢、延迟开花、发育不良和叶片黄萎等症状(Zabaleta等1994)。水稻中报告有6个基因序列有可能编码质体Cpn60, 包括3个Cpn60α和3个Cpn60β, 其中Cpn60α1对rbcL折叠至关重要(Kim等2013a)。Jiang等(2014)报告在水稻苗期低温白化高温转录的突变体中, TCD9编码Cpn60α对于早期叶绿体发育具有重要作用。

3.2 高温下RCA与Cpn60的结合

高温影响RCA结构和分布, 在Mg^{2+}和ATP存在时, 每个RCA聚合体的分子质量超过600 kDa, 而热失活的RCA分子质量可达1 400 kDa (Wang等1993), 表明RCA多肽形成了更高级的聚合体(Crafts-Brandner 1997), 同时RCA从可溶性组分向不可溶组分转移。有研究表明RCA受高温胁迫后的重新分布与RCA和类囊体的结合有关(Rokka等2001)。但Salvucci等(2001)发现使用盐处理和去污剂处理类囊体膜, 无法使RCA重新溶解; 体外实验中, 当温度超过RCA最适温度、反应介质不存在类囊体膜结构物时, RCA能够沉淀, 这说明受高温胁迫时RCA在植物体内分布的变化不是通过RCA和类囊体膜的结合实现, 而应该是自身热聚合所致。通过在烟草中超表达菠菜甜菜碱基因和水稻中超表达景天庚酮糖-1,7-二磷酸酶基因证明了可以降低高温下RCA热聚合而提高其光合的耐热性(Yang等2005; Feng等2007)。玉米叶绿体蛋白延长因子EF-Tu提高耐热性的原因是其能够减少RCA的热聚合(Rao等2004; Ristic等2004)。但也有报道类囊体上RCA含量在热胁迫和正常条件下及生长不同周期时都存在变化, 高ATP含量和pH值会从类囊体

膜上解离下更多的RCA, 高温胁迫下热聚合并不能完全解释类囊体RCA含量的变化(Chen等2010)。

Salvucci (2008)通过研究热胁迫条件下的拟南芥, 发现体内RCA和Cpn60β有相互结合的现象, 且仅在热胁迫条件才相互结合, 推测Cpn60β很可能在避免RCA热失活过程中起保护作用, 从而维护RuBisCO活性达到光合热适应的作用。王盾(2010)利用水稻热胁迫材料, 通过免疫共沉淀技术, 用RCA抗体钓到Cpn60蛋白, 该蛋白只有在高温条件下才能通过RCA抗体分离到, 恢复常温则分离不到。利用串联质谱技术对高温条件下与RCA特异性结合的蛋白进行了进一步研究, 发现Cpn60α和Cpn60β确实与RCA存在结合作用, 此外利用免疫共沉淀和双分子荧光技术验证了Cpn60β和RCA在体内存在互作, 转基因过表达Cpn60β1株系提高了高温下水稻光合速率, 与RCA结合的Cpn60β1蛋白明显增多(陈跃2015; 周丽2015)。

4 展望

RCA作为调节RuBisCO活性的关键酶, 历经30余年研究, 已取得不少成果, 但RCA在植物体内所起的作用还没有完全厘清。为什么要保留大小同工型? 其在应对逆境胁迫中的作用到底是什么? 这些问题均有待进一步解答。

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The role of ribulose-1,5-bisphosphate carboxylase/oxygenase activase in resistance of plant to abiotic stresses

CHEN Hou-Ming, CHEN Yue, WANG Dun, JIANG De-An*

State Key Laboratory of Plant Physiology and Biochemistry, College of Life Sciences, Zhejiang University, Hangzhou 310058, China

Abstract: Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) activase (RCA) has attracted more and more attention since it is discovered. It becomes one of the most important fields of photosynthesis research to study enzymology characteristics, activation of Rubisco both *in vivo* and *in vitro*, regulation of photosynthetic rate and RCA activity, gene and molecular structure, antisense, and overexpression. In this paper we mainly review the molecular structure, the relationship between RCA and crop photosynthesis and, especially, the role of RCA in resistance of plant to abiotic stresses.

Key words: ribulose-1,5-bisphosphate carboxylase/oxygenase activase; photosynthesis; abiotic stress

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*Corresponding author (E-mail: dajiang@zju.edu.cn).