

植物内质网小分子量热激蛋白的生物学功能

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摘要 介绍了植物 ERsHSP 的结构特点、分子伴侣活性及逆境抗性等生物学功能, 并展望了该领域今后的研究方向, 为深入研究植物的逆境胁迫机制以及分子水平的作物育种提供了新思路。

关键词 内质网小分子量热激蛋白; 分子伴侣; 逆境抗性; 内质网应激

中图分类号 Q946.1 **文献标识码** A **文章编号** 0517-6611(2017)30-0007-03

Biological Functions of Plant Small Heat Shock Protein in Endoplasmic Reticulum

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Abstract The structure of plant endoplasmic reticulum-localized small heat shock protein(ERsHSP) characteristics, biological function as molecular chaperone activity and adversity resistance were reviewed, and research direction in this field was analyzed, which lay a foundation for further research of plant adversity stress mechanism, and provided a new way for molecular plant breeding.

Key words ERsHSP; Molecular chaperone; Stress resistance; ER-stress

热激蛋白(HSP)是生物体在高温、低温、干旱等胁迫下合成的一类应激蛋白, 是保守的核基因家族^[1]。根据分子量的大小分为 HSP110、HSP90、HSP70、HSP50 和小分子量热激蛋白(sHSP, 17~30 kD)。sHSP 在植物中种类多, 分布广。根据分布和结构特征常分为细胞质 I 类 sHSP (Class I sHSP)、细胞质 II 类 sHSP (Class II sHSP) 和细胞器 sHSP, 其中细胞器 sHSP 分为叶绿体 sHSP (CPsHSP)、线粒体 sHSP (MTsHSP) 和内膜 sHSP (一般指的是内质网 sHSP, 即 ERsHSP)^[2]。后来在拟南芥中又发现了一类新的胞质定位的 sHSPs 家族——Class III^[3]。

ERsHSP(Endoplasmic reticulum-localized sHSP)是定位于内质网中的一类 sHSP。内质网占细胞膜系统的 50% 左右, 是蛋白质、脂质合成的主要场所。在逆境胁迫下细胞的最初反应是内质网应激^[4]。内质网应激发生时, 蛋白质异常折叠积累, 脂类合成受阻, 从而导致生理活动失衡。但同时可以激活内质网内的 HSP 执行分子伴侣功能, 降低次生伤害。GRP78 或 Bip 是内质网中 2 种标志性 HSP, 在异常蛋白质的重新折叠和装配过程中发挥分子伴侣作用。Cooper 等^[5]于 1987 年首次报道热激处理的玉米中表达出 ERsHSP, 1990 年 Sticher 等^[6]发现热激处理大麦, 诱导表达的 ERsHSP 对内质网中的标志性酶起保护作用。随后又在高温处理的豌豆、番茄、拟南芥以及低温胁迫的马铃薯等多种植物中检测出 ERsHSP^[7-11]。逆境是造成作物减产和限制种植广度的主要因素, 研究证实 ERsHSP 的表达与植物逆境抗性密切相关。笔者介绍了 ERsHSP 的生物学功能, 以期为深入研究植物的逆境胁迫机制以及分子水平的作物育种提供参考。

1 ERsHSP 的结构特点

HSP 是很保守的蛋白质之一。即使是原核生物和真核生物之间, 同分子量 HSP 的同源性也较高。不同植物相同种

类的 HSP 同样具有较高的同源性, 同种生物不同类型的 HSP 的同源性则较低^[12], 但 ERsHSP 与 Class I sHSP 的同源性相对高些^[7]。sHSP 的氨基酸序列包括可变 N 端、保守的 C 端结构域(即 α -结晶蛋白域)和自由的 C 端延伸部分(图 1)^[13]。研究者通过对 5 种 sHSPs (Class I sHSP、Class II sHSP、CPsHSP、MTsHSP 和 ERsHSP) 的氨基酸序列进行同源性分析, 发现与其他 sHSP 相同, ER-sHSP 的 C 末端也共有一段保守热激区域, 具有 -PGL 和 -VGL 2 个基序, 保守区之间是氨基酸数目可变的亲水区^[2], 这 2 个基序是高度保守的, 表明 ER-sHSP 应该与其他 sHSP 一样, 在植物体内担任分子伴侣的角色。sHSP 之间分子量的差异主要由于在 N 端 WDPF 域和 α -结晶蛋白域之间的片段长度不同, 以及 C 端延伸片段的长度不同造成的^[14]。

不同植物的 ERsHSP 氨基酸序列分析表明, ERsHSP 除了 C 末端的 2 个保守区之外, N 末端还有多个同源序列, 可能赋予 ERsHSP 特别的功能^[15]。另外, ERsHSP 的 C 末端含有一段四肽残基, 与内质网滞留序列 KDEL、HDEL 和 KNEL 类似^[16-18], 可能与 ERsHSP 的特殊定位有关。

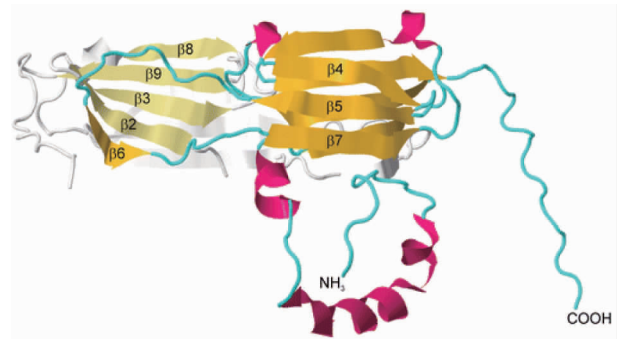


图 1 小麦 sHSP16.9 二聚体的 X 射线结构示意图

Fig. 1 Structure schematic of X-ray of wheat sHSP16.9 dimer

2 ERsHSP 的分子伴侣功能

热激蛋白是最早被发现的一类分子伴侣, 在高温胁迫时和恢复期, 检测到 15~18 kD sHSP 在细胞质和细胞器之间往返, 执行分子伴侣的功能, 减少高温伤害, 修复被损伤的蛋白

质^[18]。研究表明,sHSP的C末端的 α -结晶蛋白结构域与其分子伴侣活性密切相关^[19-21],在多种胁迫条件下可以诱导合成sHSP,与相应底物蛋白质结合,使其构象发生相应改变,从而阻止底物蛋白质的异常积聚,保持了蛋白质合成的正常进行^[22]。因此,sHSP与植物的胁迫抗性密切相关。ERsHSP作为sHSP家族成员之一,也具有分子伴侣功能。

细胞中的蛋白质合成有大约1/3在内质网中进行,迄今为止只在植物中检测出ERsHSP。研究表明,内质网中过量表达BiP(HSP70同系物)可以恢复细胞在胁迫条件下蛋白质合成的速率,从而提高生物体胁迫抗性。由于sHSP结合底物更加灵活多样,推测过量表达的ERsHSP可以和其他分子伴侣一起帮助蛋白质重新正确折叠^[22],减少胁迫下内质网中变性蛋白的积累,减少逆境对蛋白合成的影响,所以具有更重要的分子伴侣作用^[23-24]。Mamedov等^[25]证实ERsHSP(LeHSP21.5)可以在活体外有效阻止可溶蛋白的热变性。在拟南芥中细胞质小分子量热激蛋白HSP17.8可以作为分子伴侣帮助合成的质体外膜蛋白(AKR2A)到达正确位点^[26],叶绿体小分子量热激蛋白HSP21和pTAC5互作,是质体发育的必要条件^[27];在非生物胁迫下,大豆中内质网小分子量热激蛋白PvNod22可以阻止低温下内质网中变性蛋白的积累,从而保持内质网的稳态^[28]。大多数sHSP的分子伴侣活性不依赖ATP,但被依赖ATP的Hsp70/DnaK激活^[29],至于ERsHSP与其他分子伴侣(如BiP)的关系、互作位点以及信号转导途径等仍需进一步研究。

3 ERsHSP与植物的抗性

ERsHSP作为sHSP家族成员,具有分子伴侣活性,在逆境条件下帮助蛋白质重新正确折叠及复性,从而保证了植物体内多种生理生化反应的正常进行,与植物的耐热性、抗冷性的提高等密切相关。

3.1 ERsHSP与植物的耐热性 关于高温热激诱导ERsHSP的产生并提高植物耐热性的研究报道较多。研究者在豌豆幼苗中进行免疫印迹试验,发现PsHSP22.7基因在室温(21℃)时不表达,在热激(40℃)时迅速积累,表明该基因可能与植物叶片的耐热性相关^[9]。刘箭等^[30]的Northern杂交试验表明,随着温度的升高番茄中ERsHSP(LeHSP21.5)基因的表达量增加,并且在不同组织中表达的起始温度不同,在叶中是36℃时开始表达,在花中是32℃,ERsHSP的表达量与植物的耐热能力相关,表明花的耐热性低于叶。在拟南芥中热激产生的AtHSP22也可提高耐热性^[7]。

3.2 ERsHSP与植物的耐冷性 长期研究发现sHSP除了抵御高温外,对低温胁迫下的植物也具有保护作用。Sabehat等^[31]将番茄果实热激(38℃)48h后,再低温(2℃)处理,与未经过热激的对照相比,发现热激后的果实抗冷性提高,其效果可持续21d。在冬季低温条件下,桑树皮层薄壁细胞中的内质网形态会发生变化,从潴泡状变成小囊泡,同时检测有大量ERsHSP,从而提高抗寒能力,WAP27和WAP20明显增加^[32]。低温也能诱导马铃薯块茎中C119基因表达,ERsHSP的表达与植物的抗寒能力密切相关^[10]。赵春梅

等^[33-34]将ERsHSP基因导入番茄,MDA、电解质外渗及Fv/Fm等生理指标表明,组成性表达ERsHSP的转基因番茄的抗冷性优于野生型或转空载体,证实了过量表达ERsHSP的转基因番茄植株具有较强的抗冷能力。

内质网是脂类合成的主要场所。Lyons^[35]认为低温首先伤害植物生物膜的一类脂分子。膜脂不饱和度越大,膜脂相变温度就越低,从而有利于保持生物膜在低温时的流动性,维持正常生理活动。组成性表达的ERsHSP有助于保持各种脂类合成酶的活性,从而保证了类脂分子(包括低相变温度的脂类)的正常合成,提高植物的抗寒性^[33]。对Synechocystis PCC6803中的HSP17研究表明,sHSP可以调节膜脂的多态性。稳定生物膜的液晶态,在低温逆境温度条件下对膜脂的流动性具有一定的保护作用。体外试验表明,保守的sHSP的 α -晶体结构区域可以结合于质膜并且合成磷脂泡状体,帮助变性蛋白重新正确折叠^[36-37]。在温度逆境条件下向离体的类囊体膜中添加外源叶绿体HSP21对PS II有一定的保护作用^[38]。抗冷植物的不饱和脂肪酸含量高,相变温度低,所以能在低温下保持生物膜的流动性来维持正常生理活动。过量表达叶绿体小分子量热激蛋白CaHSP26可以提高低温胁迫下烟草生物膜的不饱和度,减轻低温造成的光抑制^[39-40]。ERsHSP可以通过脱落酸途径提高植物耐冷性^[41]。过量表达甘油-3-磷酸酰基转移酶基因使植物细胞内磷脂酰甘油不饱和和脂肪酸增加,提高了番茄的低温抗性^[42],表明低温胁迫时植物体可能通过某些途径增加生物膜脂不饱和度,抵抗低温伤害。

ERsHSP在番茄中过量表达,在低温条件下可以降低MDA的积累,减少电解质外渗,表明ERsHSP可以保护生物膜^[33],但是这种保护作用是否可涉及其他的细胞器膜系统,以及ERsHSP所起的具体作用还需要深入研究。

4 ERsHSP与ER-Stress

内质网是重要的细胞器,很多的蛋白质(如分泌蛋白、跨膜蛋白和内质网驻留蛋白等)需要进入内质网腔内进行翻译后的修饰、折叠和寡聚化,才能形成正确的构象。逆境会导致未折叠的异常蛋白增多,超出内质网的处理能力时,就引起ER-Stress,随即内质网会启动未折叠蛋白反应(UPR),UPR信号通路可以诱导内质网分子伴侣的表达,帮助蛋白质进行折叠和运输、降解冗余的蛋白质,并且控制分泌型蛋白进入内质网的数量。ERsHSP是内质网分子伴侣中的重要成员,内质网分子伴侣还包括葡萄糖调节蛋白家族成员、蛋白质二硫键异构酶和类凝集素分子伴侣等^[14]。前期的研究表明,ERsHSP具有特殊的结构特点,在ER-Stress中能够起到保护作用^[33]。衣霉素处理14d后,ERsHSP的转基因番茄仍然长势良好,而野生型和转空载体的植株叶子萎缩,表明ERsHSP的积累可以减轻ER-Stress。研究表明,ERsHSP减少了其他内质网分子伴侣的诱导表达,可能直接参与了UPR信号的调控^[34]。

植物UPR信号通路除了诱导内质网分子伴侣的表达以外,还涉及蛋白酶的降解途径。内质网定位的碱性亮氨酸拉

链转录因子是 UPR 信号通路中的感受蛋白。在拟南芥中已鉴定出 3 种碱性亮氨酸拉链转录因子。其中, AtbZIP17 是由盐胁迫诱导激活^[43], 与植物耐盐性相关。AtbZIP28 受衣霉素诱导被水解并释放其 N 端区域, 随后从内质网至细胞核中, 激活 ER - Stress 应答基因的表达^[44]。AtbZIP60 受衣霉素和二硫苏糖醇等的诱导激活^[45]。

5 展望

ERsHSP 的研究起步比细胞质 sHSP 和 CPsHSP 晚。ER-sHSP 作为内质网分子伴侣中的重要成员, 可以减轻 ER - Stress, 从而降低了 UPR。近年来, ERsHSP 的研究逐渐受到重视^[33-34, 41-42]。迄今为止, 人们对植物 ERsHSP 的结构和生物功能等的研究还不够全面^[46]。在温度逆境胁迫时, 植物体内有 ERsHSP 生成积累, 伴随着抗性增强, 推测其在提高植物抗性方面发挥作用。ERsHSP 对衣霉素等引起的 ER - Stress 有保护作用, 是否可以保护线粒体、叶绿体等其他的内膜系统, 是直接作用还是间接作用, 有待于进一步探讨。UPR 是一条从内质网到细胞核的信号传导途径, 其如何影响膜脂组成和状态的变化, 进而影响细胞的代谢活动和生理状态, 也有待于进一步研究。研究分子伴侣的作用机理可以揭示植物细胞抗逆性的分子机制, 并可以为分子水平上培育抗逆品种提供新思路。

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