综 述 Reviews

植物细胞壁松弛因子

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摘要: 植物细胞壁的松弛是细胞伸长必需的一个生理过程, 发生于植物生长发育的各个阶段。研究发现参与细胞壁松弛的因子有多种, 主要包括膨胀素(expansin)、木葡聚糖内转糖苷酶/水解酶(XTH)、糖基水解酶和羟基自由基(·OH)四大类。本文主要对这些细胞壁松弛因子的结构特征、作用机制及其在植物生理过程中的作用等方面的研究进展进行综述。

关键词: 细胞壁松弛; 膨胀素; 木葡聚糖内转糖苷酶/水解酶; 糖基水解酶; 羟基自由基

Plant Cell Wall Loosening Factors

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Abstract: Plant cell wall loosening is a necessary physiological process during cell expansion throughout the entire growth and development of plants. Several types of factors including expansin, xyloglucan endotransglucosylase/hydrolase (XTH), glycoside hydrolase and hydroxyl radical (·OH), have been known for functioning in this process. This review summarizes recent studies of the loosening factors in regard to their structures and characters, working mechanisms and effects on plants.

Key words: cell wall loosening; expansin; xyloglucan endotransglucosylase/hydrolase (XTH); glycoside hydrolase; hydroxyl radical

植物细胞壁是植物细胞特有的细胞结构。在很大程度上, 它决定植物细胞的形状, 控制着细胞生长的方向和速率, 并且影响各类细胞的分化和功能实现。细胞壁形成包括细胞壁的松弛与重构、细胞壁组分的合成、组装与沉积等过程。其中细胞壁松弛对植物发育有着深刻的影响, 近年来受到高度关注。

在植物发育过程中, 分生细胞分裂产生子细胞, 子细胞需经细胞扩展后再分化成各类特定细胞。在细胞扩展过程中, 已有的初生细胞壁构架需要改变以加入新物质, 因此必须有一类机制使得细胞壁基质能够发生松弛。在植物的初生细胞壁中, 纤维素和交联聚糖组成的网络结构镶嵌在果胶质多糖基质中。其中纤维素微纤丝一般不会断裂再重构, 而纤维素微纤丝与交联聚糖间氢键的破坏和交联聚糖的断裂可使微纤丝散开, 细胞壁松弛, 从而利于细胞壁新合成成分的加入。目前的研究表明参与细胞壁松弛过程的松弛因子主要有四大类, 包括膨胀素(expansin)蛋白、木葡聚糖内转糖苷酶/水解酶(xyloglucan endotransglucosylase (XET)/hydrolase, XTH)、糖基水解酶(glycoside hydrolase, GH)和羟基自由基(hydroxyl radical, ·OH)。本文主要对细胞壁松弛因子的结构特征、作用机制及其在植物生理过程中的作用进行综述, 并对相关研究方向进行展望。

1 膨胀素

在研究植物细胞“酸诱导生长”现象的过程中, Cosgrove实验室发现了能够引发起细胞壁扩展的蛋白, 并将其命名为膨胀素(McQueen-Mason等1992)。随后发现膨胀素广泛存在于植物中, 推测其可能参与植物细胞壁的松弛(Sampedro和Cosgrove 2005)。

1.1 膨胀素蛋白家族及其结构

膨胀素蛋白家族是一个庞大的超级家族(http://www.bio.psu.edu/expansins/)。对膨胀素家

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族进行系统发生树分析, 发现植物中膨胀素超级家族可分为4个亚家族, 由大到小分别命名为: α-膨胀素(EXPA)、β-膨胀素(EXPB)、膨胀素类似蛋白A (expansin like A, EXLA)和膨胀素类似蛋白B (expansin like B, EXLB) (Kende等2004)。这4个家族在拟南芥中分别有26、6、3和1个成员, 在杨树中成员分别为27、2、2和4个, 水稻中分别为34、19、4和1个(Sampedro和Cosgrove 2005)。不同亚家族的膨胀素间氨基酸序列只有20%~40%的一致性。

膨胀素蛋白分子一般有250~275个氨基酸, 由两个结构域及一段位于N端长度为20~30个氨基酸残基的信号肽组成。结构域I (domain I)由120~135个氨基酸残基组成, 它和葡萄糖苷水解酶45家族(glycoside hydrolase family 45, GH45)蛋白的催化结构域具有一定的同源性。结构域II (domain II)由90~120个氨基酸残基组成, 与禾本科Group II花粉过敏原蛋白(group 2 grass pollen allergens, G2A家族)具有一定同源性(Sampedro和Cosgrove 2005)。对膨胀素的命名, 目前遵循当一个蛋白同时具有结构域I和结构域II时才能命名为膨胀素(Kende等2004)。

Yennawar等(2006)对玉米(*Zea mays*)的EXPB1 (Zea m 1)蛋白进行三维晶体结构解析发现: EXPB1的结构域I与GH45家族的催化位点高度相似, 但是其缺少一个天冬氨酸残基, 而该残基是GH45家族酶水解活性所必需的催化碱基。EXPB1的结构域II由8个β-strands组装成2个反平行的β-片层, 形成类似免疫球蛋白的β-三明治折叠结构。这2个结构域一起形成一个长而浅的沟, 推测这可能是潜在的多糖结合位点(Yennawar等2006)。对枯草芽孢杆菌(*Bacillus subtilis*)中分离出的膨胀素类似蛋白EXLX1蛋白晶体的解析发现它也存在类似的结构(Kerff等2008)。

1.2 膨胀素的活性及其作用机制

由于膨胀素结构域I与GH45家族的催化结构域类似, 早期有人推测膨胀素可能具有糖基水解酶或内转葡糖基酶的活性(Fry 1995), 后来发现膨胀素结构域I缺少GH45家族催化所必需的一个天冬氨酸残基, 推测膨胀素不具有这类酶活性, 至今也确实未检测到它的酶活性(Yennawar等2006)。通过对禾本科Group I花粉过敏原Phl p1蛋白(EXPB亚家族蛋白)的体外重组研究, Grobe等(1999, 2002)推测膨胀素可能是C1半胱氨酸蛋白酶家族成员, 它通过降解细胞壁的结构蛋白而使细胞壁松弛。但是随后的研究认为膨胀素不具有蛋白水解酶活性(Li和Cosgrove 2001), Grobe等观察到的现象可能是由于重组Phl p1诱导了酵母内源性蛋白酶表达而引起的假象(Cosgrove等2002; Poppelmann等2002)。

虽然目前还没有发现膨胀素具有任何酶活性, 但是膨胀素确实能够促进植物细胞壁的松弛, 例如外源的EXPA和EXPB膨胀素能使热失活的细胞壁完全恢复伸展性(McQueen-Mason等1992; Cosgrove等1997)。另外, 膨胀素作用非常快速, 在加入细胞壁样品中几秒后即可诱导细胞壁伸展, 并且不影响它本身的可塑性和伸缩性(Yuan等2001)。同时, 对膨胀素松弛细胞壁的机制研究发现: EXPA可使纯纤维素组成的纸张软化而不降解纤维素, 而且能够协同地加强纤维素酶对纤维素的水解作用, 推测其弱化纤维素间的氢键(McQueen-Mason和Cosgrove 1995; Cosgrove 2000); 用浓度为2 $mol \cdot L^{-1}$的尿素打开细胞壁内的氢键, 可以模拟膨胀素对细胞壁的物理效应(Cosgrove 2005); EXPA可以使由细菌来源的纤维素和木葡聚糖组成的人工复合物瞬时的扩张(Whitney等2000), 进一步研究发现, EXPA和EXPB分别与木葡聚糖和木糖的结合活性最高, 推测体内EXPA解开木葡聚糖与其他细胞壁多糖相连的非共价键, 而体内EXPB解开木糖与其他细胞壁多糖相连的非共价键连接(Yennawar等2006)。根据膨胀素的这些特性, Cosgrove (2000, 2005)提出一个膨胀素的非酶活性作用机制模型: 膨胀素通过弱化细胞壁多糖(包括基质多糖和纤维素微纤丝)之间的非共价键, 使一些多糖在膨压驱动下滑动, 从而引起细胞壁蠕动。尽管如此, 这一模型还有待于进一步的验证与完善。对膨胀素的进一步研究将深化我们对膨胀素作用机制的认识。

1.3 膨胀素的生物学功能及其调控

膨胀素家族成员众多, 研究表明不同膨胀素基因在不同的器官、组织或细胞类型中有不同的表达, 参与植物生长发育中的多个过程, 如种子萌发、根发育、叶发育、花粉管形成、果实软化、

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器官脱落、气孔调节、植物次生生长等。同时, 膨胀素基因可以特异地应答不同的激素和环境处理(Cho和Kende 1997; Cosgrove等2002; Sampedro和Cosgrove 2005)。

在番茄(*Lycopersicon esculentum* Miller)种子萌发的过程中, *LeEXP4*在胚乳冠区域特异表达, 并且其表达水平与胚乳冠软化程度一致, 推测其参与胚根的产生(Chen和Bradford 2000)。

在根和叶发育中, 膨胀素也起着重要作用。拟南芥膨胀素*AtEXP7*和*AtEXP18*的表达可以调节根毛的起始(Cho和Cosgrove 2002), RNA干扰*AtEXP7*的表达会使根毛变短(Lin等2011); 大豆膨胀素*GmEXPB2*可促进根细胞的分化、根的伸长及根对磷的吸收(Guo等2011); 水稻膨胀素基因*OsEXPA17*和*OsEXPA20*调节根毛的伸长(Yu等2011)。番茄*LeEXP18*表达可以作为叶片起始的一个分子标记(Reinhardt等1998), 黄瓜膨胀素*CsExp1*可以调节烟草叶的起始、叶序方向的建立以及叶片形状的确立等(Pien等2001)。

烟草*EXPB*基因*PPAL*在柱头的分泌区与胎座的表皮层特异表达, 并调节花粉管侵入柱头及其在花柱中生长的过程(Pezzotti等2002)。玉米一个*EXPB*基因缺失会导致花粉易聚集, 花粉囊裂开时花粉不能很好地散布, 花粉管发育受阻(Valdivia等2009)。

果实成熟和器官脱落也需要膨胀素调节。过表达番茄*LeEXP1*基因会使果实在绿果时就变软, 而降低其表达, 成熟后的果实依然比较硬(Brummell等1999)。其他水果中也发现与果实软化相关的膨胀素基因(Rose等1997; Harrison等2001; Obenland等2003)。在拟南芥叶柄基部过表达*AtEXP10*, 可促使植株叶柄脱落(Cho和Cosgrove 2000); 西洋接骨木(*Sambucus nigra*)中, 乙烯可以特异性诱导2个*EXPA*基因在叶柄脱落区表达(Belfield等2005)。

最近发现拟南芥膨胀素*AtEXPA1*基因在气孔保卫细胞中表达, 并影响气孔开闭的难易程度。过表达*AtEXPA1*可加速光诱导的气孔张开, 反之则效果相反(Zhang等2011; Wei等2011)。

膨胀素也参与了植物次生生长。百日草叶肉细胞转分化为导管的研究中发现了一些膨胀素基因表达(Milioni等2001)。杨树中的一些膨胀素*EXPA*基因可能参与木质部的形成(Gray-Mitsumune等2004)。棉花纤维的形成过程中也可能有膨胀素参与, 在一个短纤维棉花突变体*Ligon Lintless*中多个膨胀素基因显著下调(Bolton等2009)。

另外, 膨胀素也参与一些特定植物的特定发育过程, 如在豆科植物中固氮瘤的生长(Giordano和Hirsch 2004)、黄瓜根组织中共生真菌菌根的形成(Balestrini等2005)、寄生植物的生长(O'Malley和Lynn 2000)以及"复苏植物"的脱水与复水(Jones和McQueen-Mason 2004)等。

膨胀素的表达除了受到这些发育进程的调控, 激素和环境因子也对其有很大的影响。如膨胀素基因的表达与淹水和赤霉素诱导的深水稻节间的快速生长紧密相关(Cho和Kende 1997); 酸模植物中, 乙烯可调控质外体快速酸化及膨胀素表达(Vreeburg等2005); 外源生长素可诱导松树中膨胀素的表达(Hutchison等1999); 光可调控*AtEXPA1*的表达(Zhang等2011); 干旱也可诱导膨胀素的表达(Wu等1996)。但是, 膨胀素的调控机制还有待深入研究。

2 木葡聚糖内转糖苷酶/水解酶

除膨胀素外, 研究发现细胞壁中的一类木葡聚糖内转糖苷酶/水解酶, 能催化木葡聚糖分子水解与再连接, 通过一种"分子嫁接"的机制介导细胞壁的松弛及重构(Fry等1992; Nishitani和Tominaga 1992)。

2.1 XTH家族蛋白及其结构

XTHs为多基因家族编码的一类酶, 属于糖苷水解酶GH16家族蛋白。高等植物一般含有20~60个XTH蛋白(Eklof和Brumer 2010)。按其序列特征, XTHs被分为I、II和III三大类(Campbell和Braam 1999a; Rose等2002), III类XTHs又可分为IIIA和IIIB两小类(Baumann等2007)。目前发现I类、II类和IIIB类XTHs具有显著的转糖基酶(XET)活性, 而IIIA类只表现为水解酶(xyloglucan endo-hydrolase, XEH)活性。随着植物基因组测序的展开, 更多不同物种的XTH蛋白被发现与鉴定(Yokoyama等2004; Michailidis等2009)。但是在苔藓和早期的维管植物如石松属植物中却没有发现IIIA类XTH蛋白, 推测XTH蛋白的水解酶活性是进化而来的(Baumann等2007; Eklof和Brumer 2010)。

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目前, 对具有XET和XEH两种活性的蛋白分别进行了三维结构解析。杂交杨(*Populus tremula* ×*Populus tremuloides*) Ptt-XET16-34蛋白具有XET活性, 旱金莲(*Tropaeolum majus*) TmNXG1具有XEH活性。它们的蛋白晶体结构均被解析出来, 蛋白质数据库(PDB, http://www.pdb.org)的ID分别为: 1un1和2uwa (Johansson等2004; Baumann等2007)。分析发现它们和GH16家族的成员一样都具有β-卷芯(β-jellyroll)折叠, 不同的是它们具有较宽的底物结合缝隙, 可以结合高度分支的多糖(Eklof和Brumer 2010)。XTHs蛋白的催化模体序列为(H/W/R)-(D/N)-E-(I/L/F/V)-D-(F/I/L/M)–E-(F/L)-(L/M)-G (下划线表示最常出现的氨基酸), 其中第一个谷氨酸是亲核试剂, 第二个谷氨酸是质子供体。研究发现催化位点附近的苏氨酸或丝氨酸残基的*N*-糖基化修饰对酶活性有重要影响(Kallas等2005; Van Sandt等2006)。*N*-糖基化位点在I/II类中是保守的, 但是在IIIA中不存在(Baumann等2007), 去除*N*-糖基化修饰会大大降低蛋白的稳定性并影响它的正确折叠, 另外Ptt-XET16-34和TmNXG1的C端有高度保守的半胱氨酸残基, 可以形成二硫键, 对于稳定蛋白结构也十分重要(Campbell和Braam 1999b; Kallas等2005; Eklof和Brumer 2010)。

2.2 XTHs的作用机制

对XTHs蛋白“分子嫁接”机理的研究一直是重点。研究发现, XTHs采用保持异头构型的两步置换机制(retaining mechanism)进行催化反应(图1)。当XTH与底物结合后, 首先将底物水解, 并形成一种糖基-酶的共价中间产物, 然后它再将糖基转移给多糖残基的还原端或水分子, 发生转糖基反应(XET活性)或发生水解反应(XEH活性)。

XET和XEH活性差异的基础是其蛋白结构的差异, 深入分析PttXET16-34和TmNXG1的三维结构发现: 二者的最大差异在于底物结合位点两侧的两个环结构, 即位于结合位点–1与–2亚位点的环1 (loop1)和位于+1与+2亚位点的环2 (loop2)。对该家族其他酶的蛋白序列分析发现, 环1在两类酶蛋白中没有普遍的差异, 而环2则有明显差异: IIIB类酶的环2中有两个氨基酸(XG)的插入, IIIA中有5个氨基酸(G/Y)NIIG的插入。将TmNXG1的环2截短突变产生TmNXG1-ΔYNIIG, 其环2的长度相当于PttXET16-34的短环2。酶活测定发现TmNXG1-ΔYNIIG产生了转糖基酶活性, 而水解酶活性降低。这表明环2的长度对于调控XET和XEH的活性有重要的调节作用。但由于TmNXG1-ΔYNIIG仍然具有XEH活性, 表明还存在其他潜在的调控两种酶活性的位点(Baumann等2007)。

XTH的最适底物是木葡聚糖, 最小供体为XXXGXXXG, 三维结构显示XTH活性位点有一个35 Å的缝隙, 初始可以容纳7个主链葡萄糖基(图1),

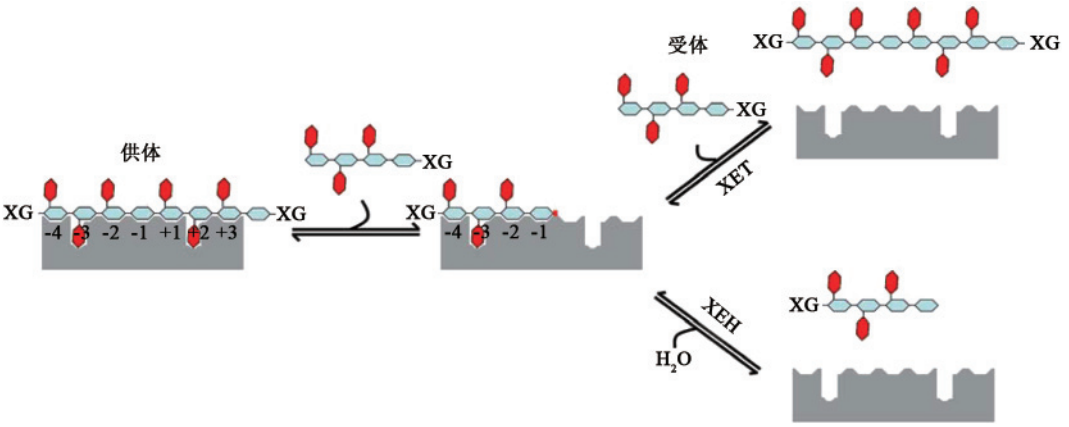

图1 XTHs催化木葡聚糖机制示意图(Baumann等2007; Eklof和Brumer 2010)

Fig.1 A schematic representation of the mechanism used by XTHs

左图: 木葡聚糖结合在XTH的–4至+3亚位点, 蓝色六边形表示葡萄糖基, 红色六边形表示木糖基, 灰色多边形表示XTH, 数字为亚位点; 中图: 酶与底物结合后, 底物被特异剪切, 产生糖基-酶中间体(红色短粗线表示共价键); 右图: 糖基-酶中间体可将糖基转移给水(XEH活性)或木葡聚糖的还原端(XET活性)。

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其中–1位非取代的葡萄糖基对酶底复合物构象的维持有重要作用, 是催化能够进行的重要条件(Davies等1998; Mark等2009)。+2亚位点的木糖基取代对于酶活性至关重要, 可能是该酶的一个重要识别位点(Saura-Valls等2008)。其他的一些半乳糖基或者岩藻糖侧链有时也会影响XET活性, 但是效果有限(Fry等1992; Maris等2009)。虽然有研究表明较长的底物能够增加转糖基活性(Saura-Valls等2008), 但是目前还没有可靠的证据表明XTH需要更长的最小供体(Baumann等2007; Ibatullin等2008; Eklof和Brumer 2010)。

另外, Hrmova等(2007)发现酵母中表达的大麦HvXET6蛋白具有低水平的异转糖基活性, 由于禾本科植物细胞壁中只含有少量的木葡聚糖而含有大量的混合链葡聚糖, 因此推测HvXET6在体内可能具有混合链葡聚糖:木葡聚糖内转糖基酶活性(mixed-linkage β-glucan:xyloglucan endo-transglucosylase, MXE)。虽然随后在一些物种如木贼属马尾草、轮藻粗提物中检测到很高的MXE活性, 但是利用一些陆地植物包括禾本科植物的粗提物却没有检测到高的MXE活性(Fry等2008; Hrmova等2009), 因此目前尚不能判断植物体内的XTH是否具有MXE的功能。

2.3 XTHs的生理作用及其调控

XTHs家族庞大且表达各异(Becnel等2006; Mellerowicz和Sundberg 2008; Miedes和Lorences 2009), 在植物的多个生长发育过程中起着重要的作用, 如根发育、花的发育、果实成熟和木质部形成等。

在拟南芥中, 每个发育阶段都有XTHs的表达(Becnel等2006)。*AtXTH18*调节根的伸长过程(Osato等2006); *AtXTH17*、*AtXTH19*和*AtXTH20*分别在根的不同部位表达, 对根伸长、根毛起始等都起着重要作用(Vissenberg等2005); *AtXTH9*的突变会导致节间变短(Hyodo等2003); *AtXTH27*则影响叶导管的产生(Matsui等2005); *AtXTH28*则可能参与雄蕊花丝发育(Kurasawa等2009)。

Miedes和Lorences (2009)对番茄果实生长和成熟中表达的10个*SlXTH*研究表明, I类的*SlXTH1*、*SlXTH*4和*SlXTH7*, II类的*SlXTH12*以及IIIB类的*SlXTH6*均与果实生长有关, 只有IIIA类中的*SlXTH5*和*SlXTH8*与果实的成熟有关。XET和XTH总活性均在果实生长过程中增加, 在果实成熟过程中下降。XTH可能对细胞壁完整性的维持有作用, 果实软化需受不同XTHs调节。

在木本植物次生壁(木材)形成过程中也有多个*XTH*基因的参与(Mellerowicz和Sundberg 2008)。如在杨树应拉木纤维细胞壁胶质层也能检测到XET活性和XTH产物; 在应拉木形成过程中, 多个*XTH*基因的表达上调, 甚至在成熟的胶质木纤维中仍能检测到XTH产物; XET活性明显地存在于靠近细胞壁S2层处, 它被认为可修复胶质层与S2层间木葡聚糖的交联, 从而维持胶质木纤维的收缩(Nishikubo等2007)。

现已发现多种激素和环境刺激可以诱导*XTH*的表达。如油菜素内酯可诱导豌豆XTH家族成员BRU1在胚轴组织的内部积累, 参与调节维管和皮层细胞的增大(Oh等1998); 生长素可诱导*SlXTH1*在番茄胚轴中表达上调(Catala等1997); 赤霉素可上调水稻*OsXTH8*表达, 可能参与细胞伸长(Jan等2004)。另外环境刺激如触摸可诱导*AtXTH22*的表达(Braam 1992), 重力可影响豌豆上胚轴中*XTH*的表达(Soga等2007), 水淹(缺氧)可以诱导转基因玉米中*wusl1005*表达XTH蛋白(Saab和Sachs 1996)等。虽然XTHs的研究已取得诸多进展, 但是其生化和遗传方面的功能仍有待进一步的挖掘。

3 糖基水解酶家族成员

除了膨胀素和XTH外, 其他GH家族的糖基水解酶也被发现参与细胞壁的松弛与重构从而影响植物多种生理过程, 如GH9家族的内切-β-葡聚糖酶(endo-β-1,4-glucanase, EGase)和GH5家族的内切-β-甘露聚糖酶(endo-β-1,4-mannase, MAN)等。

3.1 内切-β-葡聚糖酶

植物EGase属于GH9家族, 根据系统发生树分析可将植物中EGase分为α、β、γ三组, 其中α和β组则被认为是分泌蛋白, γ组为膜结合的蛋白, 也称为KORRIGAN (KOR)或者KOR类似蛋白(Libertini等2004)。根据GH9成员的结构特征也可将其分为A、B、C三类, A类酶有胞内结构域、跨膜区和催化结构域, B类酶具有信号肽和催化结构域, C类酶具有信号肽、催化结构域和一个碳水化合物结合模块CBM49 (Urbanowicz等2007)。

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EGase的潜在底物是非结晶区的纤维素和木葡聚糖, 它水解木葡聚糖来释放微纤丝从而诱导细胞壁松弛(Trainotti等1999)。EGase通过形成倒位异头构型的一步置换反应(inverting mechanism)切割两个非取代葡萄糖基间的β-1,4-糖苷键, 达到水解多糖的目的(Gebler等1992), 这类酶只具有水解酶的活性而不具有糖基转移酶活性(Eklof和Brumer 2010)。与膨胀素完全不同, 体外检测发现EGase不具有延伸分离的细胞壁的能力(Molhoj等2002), 只在真菌中发现一个GH12家族的EGase (Cel12A)有这种活性(Yuan等2001), 但是植物中没有GH12家族的EGase, 因此Cosgrove (1999)认为它们可能通过改变细胞壁的的粘度和/或多孔性间接促进细胞壁的膨胀。

EGase参与了多个植物生理过程, 包括细胞壁的合成与修饰、细胞的伸长与分化、胞浆移动、器官脱落和果实成熟等(Rose和Bennett 1999)。如KOR参与纤维素的合成(Lane等2001; Sato等2001), 推测它可能具有切割植物纤维素合成的引物谷甾醇-β-葡萄糖苷(Peng等2002)等作用。杨树PopCel1和PopCel2分别为胞外EGase蛋白和壁结合EGase蛋白, 它们在蔗糖存在时受生长素诱导并对叶的伸长有重要作用(Ohmiya等2000); 在拟南芥中的同源蛋白Cel1也同样影响叶的生长(Tsabary等2003)。另外, EGase也参与了果实成熟过程中木葡聚糖的周转(Rose和Bennett 1999; Trainotti等1999)。EGase参与和细胞壁松弛与重构相关的生理过程, 还有待于深入研究。

3.2 内切-β-甘露聚糖酶

植物内切-β-甘露聚糖酶属于GH5家族, 和XTHs类似, 该家族成员利用保持异头构型的两步置换机制催化β-(1,4)-甘露糖苷键连接的甘露糖骨架, 可能具有水解和/或转糖基的功能(Schroder等2009)。体外研究发现, 在有甘露聚糖来源的寡糖存在时, 番茄的LeMAN4a除具有内切-β-甘露聚糖酶活性, 还具有甘露聚糖转糖基酶活性(Schroder等2006), 这些暗示我们MAN蛋白可能通过与XTHs类似的机制促进细胞壁松弛与重构, 只是对细胞壁多糖的选择性不同。

植物中, MAN在多种组织或细胞中表达(Yuan等2007), 对植物发育过程起着重要作用。如LeMAN1/2/3在番茄种子发育过程中起作用(Bewley等1997; Nonogaki等2000; Gong和Bewley 2007); LeMAN4a在番茄果实成熟中起作用(Bewley等2000); LeMAN5在番茄花粉囊及花粉的发育中起作用(Filichkin等2004)。我们课题组在杨树中发现一个木质部中特异表达的内切-β-甘露聚糖酶, 它不但可以促进导管细胞的伸长与膨胀, 还调控导管的发育(待发表)。关于植物内切-β-甘露聚糖酶参与的生理过程还有待进一步的挖掘。

3.3 其他GH家族的水解酶

根据GH家族酶的催化机制和植物细胞壁中多糖组分的差异, 我们推测其他一些GH家族的多糖水解酶也可能参与细胞壁的松弛。有些酶利用保持异头构型的两步置换机制催化反应, 可发挥与植物XTH及MAN类似的功能, 如GH5家族的葡聚糖酶和半乳聚糖酶, GH10家族的木聚糖酶以及GH17家族的β-1,3-葡聚糖酶。一些物种中检测到的MXE活性(Hrmova等2009)也有可能由这些酶来完成。另外也有一些采取与EGase类似的形成倒位异头构型的一步置换反应机制, 如GH28家族的半乳糖醛酸酶, 它们可水解基质多糖来松弛细胞壁。研究这些酶对于细胞壁基质多糖水解与再连接的活性, 可以让我们更全面地了解细胞壁松弛及重构的机制。

4 羟基自由基

羟基自由基(·OH)是一种具有高度活性的活性氧物质, 研究表明细胞壁中产生的羟基自由基可以通过非酶促的方法移除底物的氢原子来剪切壁多糖, 从而使细胞壁松弛、刺激细胞扩大(Fry 1998; Chen和Schopfer 1999)。

植物内源羟基自由基产生方式主要有两种。一种是Fenton反应, 即金属离子催化过氧化氢产生羟基自由基(Schopfer 2001; Schweikert等2002)。研究表明细胞壁结合的二价铜离子和抗坏血酸盐或超氧化物反应可以生成一价铜离子和过氧化氢, 它们通过Fenton反应产生羟基自由基, 进而松弛细胞壁(Fry等2002)。这个假说得到一系列实验的支持, 如在洋葱根中施加外源抗坏血酸能促进细胞膨胀; 施加外源Cu^{2+}可使烟草花粉管伸长; 过氧化氢可以促进梨(*Pyrus communis*)果实的软化(Fry等2001, 2002)。另一种途径是细胞壁中过氧化物酶

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(POD)催化过氧化氢和超氧阴离子产生羟基自由基, 且过氧化氢和超氧阴离子的产生需要NAD(P)H的帮助(Schopfer等2002; Liszkay等2003)。这个假说也有一些证据的支持: POD大量的存在于生长的玉米胚芽鞘细胞壁中; 外加NADH可以提高玉米胚芽鞘中过氧化氢和超氧阴离子的产生; 在氧气和还原剂NAD(P)H存在的条件下, POD在体外可以产生羟基自由基; POD抑制剂可以抑制生长素诱导的玉米胚芽鞘的伸长等(Liszkay等2003)。另外研究发现植物细胞中羟基自由基的产生受到生长素调节(Schopfer等2002)。

研究发现羟基自由基对细胞扩展的贡献很小, 只能导致细胞扩展1%, 而“酸生长”扩展度能达到40%~100%, 更重要的是自由基对于多糖的破坏没有选择性, 这样的调控是不可控的(Cosgrove 2005)。然而羟基自由基的确会在体内特定的部位参与细胞壁的松弛, 如梨果实的成熟(Fry等2001)、水芹(*Lepidium sativum*)种子的萌发及玉米胚芽鞘的伸长过程(Muller等2009)等。因此植物内源羟基自由基对细胞壁松弛的确切作用机制还有待研究。

5 总结与展望

细胞壁的松弛与重构伴随植物整个生长发育过程, 多个细胞壁松弛因子及其庞大的家族成员参与其中, 不同类型的因子通常协同发挥作用(图2)。膨胀素超级家族不具酶活性, 通过打断束缚微纤丝的非共价键而允许细胞壁压力释放, 促进细胞伸展; XTH通过切割和/或重连木葡聚糖而促进细胞壁松弛与重构; EGase和其他的水解酶降解纤维素微纤丝的非结晶区或束缚微纤丝的交联聚糖, 从而增加细胞壁的伸展性, 促进细胞生长; 羟基自由基在植物体内被诱导产生后, 通过非酶促反应方式切割细胞壁多糖, 从而使细胞壁松弛。

然而, 细胞壁松弛因子的研究仍然存在许多困惑有待解决, 如这些松弛因子家族成员的底物特异性、作用部位的特异性以及它们之间相互协作的作用方式仍然不清楚。它们除了通过松弛与重构细胞壁的方式调控植物的发育进程外, 是否还有其他的方式? 它们如何受激素、环境胁迫或生物胁迫等因素的调控以及调控通路之间是否具有交叉? 深入研究并解决这些问题将有利于我们更好地了解植物细胞壁松弛过程, 从而深入地认识植物的生长发育过程。

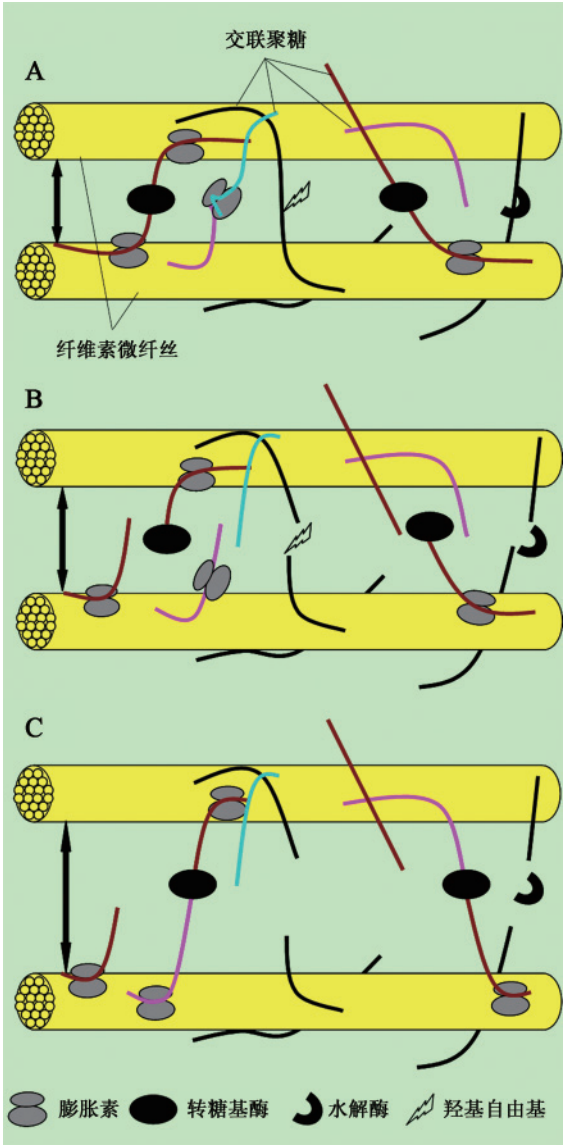

图2 植物细胞壁松弛因子的作用模型

Fig.2 A working model for plant cell wall loosening factors

渗透压驱动的微纤丝的分离通过系在其上的交联聚糖的松弛而得以实现。这个过程可能有多种细胞壁松弛因子协同完成(A)。膨胀素破坏多糖间的氢键; 转糖基酶水解聚糖, 并将部分链重连至另一条链的非还原末端; 水解酶和羟基自由基分别特异及非特异的切割交联聚糖, 使得细胞壁暂时性松弛(B)。渗透压产生的纵向的应力(箭头所示)使得微纤丝散开, 松弛的聚糖又被拉紧(C)。不同颜色的曲线表示不同的交联聚糖。

细胞壁松弛因子具有潜在生物工程和其他应用价值。如通过基因工程手段可以定向表达或调控特异植物细胞壁松弛因子, 从而有效修饰植物

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细胞壁的结构和物理特性，调节植物体的生长发育。另外，一些因子本身可以用于对细胞壁材料进行松弛处理，提高细胞壁中纤维素的分离和生产效率，在纤维材料和生物能源的生产中具有广泛的应用价值。

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