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花药绒毡层发育及调控的分子研究进展

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摘要 植物雄性生殖发育过程中, 花粉(雄配子体)形成是授粉受精、结实产果的基础. 高等植物雄蕊中, 花粉产生于花药. 花药由多层细胞构成, 绒毡层作为花药壁的最内层, 直接为花粉形成提供必需的营养、原料和能量; 反之, 绒毡层发育受阻会导致花粉败育即为雄性不育表型. 在农业生产上, 雄性不育是重要的农艺性状, 也是杂交制种的基础. 因此, 开展花药绒毡层发育及调控的分子机理研究, 不仅拓展对植物雄性生殖发育领域的理解, 同时为创制新型雄性不育系、提升中国农作物设计育种能力提供必要的理论依据. 花药发育中, 绒毡层细胞依次经历3个主要阶段: 细胞形成、细胞特化和程序性死亡. 结合近年来的研究进展, 本文围绕绒毡层发育各阶段的关键生物学事件, 重点介绍相关基因的生物学功能及分子通路, 由此系统阐述绒毡层发育以及与花药各层协同互作等一系列生物学过程的分子基础.

关键词 绒毡层, 细胞形成, 细胞特化, 花粉壁建成, 花药壁, 雄性不育

粮食安全关系到中国经济发展的大局, 是决定中国社会能否和谐发展的物质基础. 农业生产上, 作物雄性不育是提升杂交制种效率、扩大作物产量的关键性状[1~3]. 然而, 由于目前能够广泛应用的雄性不育遗传位点十分有限, 限制了杂种优势的利用效率. 花药是植物的雄性生殖器官, 是花粉发育的场所; 花药发育异常直接导致花粉败育, 造成雄性不育. 因此, 对于花药与花粉发育分子调控机制的解析是创制新型雄性不育系、突破种质资源瓶颈问题的重要途径.

高等植物花药由四个药室组成; 每个药室壁(又称花药壁)由四层体细胞构成, 从外至内依次为表皮层、内皮层、中层、绒毡层(tapetum). 一直以来, 绒毡层始终是花药四层壁细胞中的研究焦点, 对其最早的文献记载可追溯至19世纪[4,5]. 早期研究集中开展了对绒毡层细胞形态的观察和描述, 孢粉学家根据这些结果将绒毡层分为两大类型: 变形型和分泌型. 变形型绒毡层的主要特点是随着细胞壁降解, 原生质体融合成合胞体并直接包裹小孢子. 分泌型绒毡层的主要特点是无论细胞壁是否降解, 绒毡层细胞的位置维持不变, 小孢子则自由释放于药室腔中[6~8]. 据不完全统计, 被子植物门中分泌型绒毡层为主要类型且88%属于双子叶植物; 而在单子叶植物中, 大部分经济作物如: 水

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稻、玉米、小麦和大麦中的绒毡层细胞也均为分泌型[9], 因此, 本文重点以分泌型绒毡层细胞为例.

20世纪初, 随着透射电子显微镜技术的发展, 绒毡层发育过程被明确(图1): 花药发育早期, 绒毡层细胞形成(tapetal cell identify)并紧邻于小孢子母细胞; 中期, 绒毡层细胞特化(tapetal cell re-differentiation), 其细胞质浓密、细胞器丰富, 电子致密度较深的物质被大量合成并转运至小孢子表面, 组成花粉壁; 后期, 绒毡层细胞程序性死亡(programmed cell death, PCD), 分解物填入花粉壁缝隙, 完成花粉壁结构. 由此推测, 绒毡层主要为小孢子/花粉提供营养和原料物质, 尤其为花粉壁形成供给必要的前体物质[10~12]. 20世纪后半叶, 依托于分子生物学的迅猛发展, 利用特异启动子将细胞毒素基因表达于绒毡层, 有缺陷的绒毡层会直接导致小孢子缺失花粉壁、植株雄性不育[13,14], 该结果不仅印证了早期对于绒毡层功能的推测, 更强有力地证明成熟花粉形成依赖于绒毡层的正常发育.

进入21世纪, 通过遗传学、分子生物学和生物化学的手段, 大量表达于绒毡层的关键基因及其涉及的分子网络已被逐步解析[15~18](表1)[19~79]. 其中, 在拟南芥绒毡层发育不同时期表达的5个关键转录因子DYT1 (DYSFUNCTIONAL TAPETUM 1)、TDF1 (DEFECTIVE IN TAPETAL DEVELOPMENT AND FUNCTION 1)、AMS (ABORTED MICROSPORES)、MS188 (MALE STERILE 188)和MS1 (MALE STERILITY 1)被相继克隆[26,71~73,80,81]. 进一步生化数据表明, DYT1-TDF1-AMS-MS188-MS1形成了一条转录激活通路[30,75,82,83], 对绒毡层发育起到重要的调控作用. 与此同时, 这些绒毡层转录因子的同源基因在作物中也形成了类似的保守转录调控通路[84,85]. 例如, 水稻中为OsUDT1-OsTDF1-OsTDR-OsMS188-OsPTC1[86~90]、玉米中为ZmMs32-ZmMs9-ZmbHLH51-ZmMYB84-ZmMs7[91~94]. 此外, 多个转录因子家族包括bHLH、ARF、AHL和LBD 也参与到上述通路中且不同的转录因子之间还能够通过前馈激活或反馈抑制的作用模式对下游靶基因的表达进行严格调控[24,25,74,95~99](图1). 近期, 多篇综述也集中对这些转录调控通路进行了阐述、比较和总结[100~102], 揭示了转录调控网络不仅能够确保绒毡层有序地完成自身发育进程; 同时精准地控制绒毡层功能来匹配花粉生长的要求, 由此保证成熟花粉产生、为后续受精和胚胎发生奠定基础[103]. 基于以上, 本文以花药绒毡层发育进程为脉络, 综合近期研究成果, 系统地阐述绒毡层发育的分子基础, 希望能为该领域研究提供理论参考和研究思路.

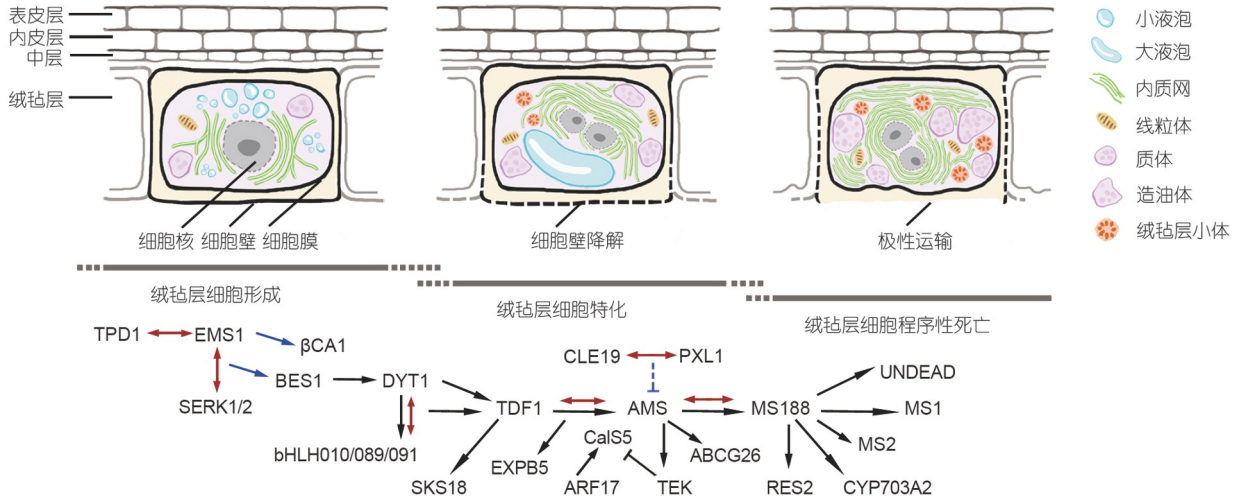

图 1　绒毡层发育的分子调控模式图. 绒毡层是四层花药壁的最内层, 其主要经历细胞形成、细胞特化和细胞程序性死亡三个发育阶段. 不同的信号通路、转录通路和蛋白质翻译后修饰参与调控拟南芥绒毡层细胞的发育. 黑色线条代表转录激活/转录抑制; 红色双箭头代表蛋白互作; 蓝色线条代表磷酸化修饰

Figure 1　A proposed model of the molecular regulatory pathways in the tapetum development. The tapetum is the innermost layer of the 4 cell-layered anther wall, which mainly undergoes cell identify, cell re-differentiation and PCD. The different signaling pathways, transcriptional pathways and protein translational modifications are involved in the regulation for the tapetum development in *Arabidopsis*. The black line indicates positive/negative transcriptional regulation; the double red arrow indicates physical interaction; the blue line indicates protein phosphorylation

1 绒毡层细胞形成

拟南芥花药发育根据其细胞学特征被分为14个时期, 第1~5期主要进行花药形态建成[104,105]. 据观察, 构成花药的多层细胞均来源于花分生组织区的三层花药原基(L1~L3). 通过垂周和平周分裂, L1层细胞形成表皮层; L3层细胞形成连接细胞和导管组织; L2层则逐步分化为造孢细胞和初生壁细胞; 至第5期, 造孢细胞形成小孢子母细胞; 初生壁细胞经次生壁细胞形成内皮层, 中层和绒毡层. 前期研究表明, 绒毡层细胞形成受到细胞与细胞之间信号网络的严格调控, 近期研究成果具体揭示了富含亮氨酸重复序列类受体激酶(leucine-rich repeat receptor-like kinase, LRR-RLK)介导的多肽信号转导通路对于绒毡层细胞形成的重要作用.

1.1 EMS1-TPD1-SERK1/2信号转导途径

早期研究发现, *EMS1*(*EXCESS MICROSPOROCYTES 1*)/*EXS*(*EXCESS MICROSPOROCYTES 1*)编码一个跨膜受体激酶(LRR-RLK)[106]; *TPD1*(*TAPETUM DETERMINANT 1*)编码一个富含半胱氨酸的短肽[19]; *ems1*和*tpd1*突变体中绒毡层细胞均缺失, 取而代之产生了更多的小孢子母细胞. 基因表达模式显示: 花药发育第4期, *TPD1*和*EMS1*均表达于造孢细胞和次生壁细胞. 第5期, *TPD1*主要在小孢子母细胞中表达; *EMS1*则更集中在绒毡层细胞中表达. 上述结果提示了*EMS1*和*TPD1*可能共同参与调控绒毡层细胞的形成.

之后, 国内外团队通过细胞学、遗传学和生物化学等手段更详尽的解析了EMS1-TPD1信号转导通路对于绒毡层细胞形成的调控机制: 花药发育第4期, EMS1蛋白定位于由次生壁细胞分化而来的绒毡层前体细胞和中层细胞, 造孢细胞产生TPD1小肽并向旁邻的绒毡层前体细胞分泌. 进入花药发育第5期, TPD1小肽必须依赖于EMS1由小孢子母细胞转移至绒毡层前体细胞的细胞膜上, 随后EMS1磷酸化开启信号通路; 第5期末, 绒毡层细胞形成, 并且通过垂周分裂进行扩增[20,107,108]. 此外, 多个遗传学证据进一步表明TPD1和EMS1蛋白表达模式的重要性[20,109]: (1) 异位表达TPD1蛋白, 不仅诱使绒毡层旁邻细胞出现绒毡层特异基因*A9*的表达信号, 甚至会造成花药内皮层的异常分层. (2) 利用液泡分选信号将TPD1错误转运至液泡, 绒毡层前体细胞无法正常发育. (3) 利用*A9*启动子大量表达*EMS1*会诱导绒毡层前体细胞分化出多层.

植物体细胞胚胎发生类受体激酶SERKs (somatic embryogenesis receptor-like kinases)是LRR-RLK第二亚家族, 拟南芥5个SERKs成员作为共受体参与的多条信号转导通路调控了根生长发育、气孔模式建成、花器官脱落、维管束发育、细胞死亡和先天免疫等诸多生物学过程[110]. 针对花药发育的研究, 发现*SERK1*和*SERK2* (*SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE 1/2*)在第4~5期的表达模式类似于*EMS1*. 表型分析显示, SERK1和SERK2蛋白的单突变体育性均不受影响, 而*serk1 serk2*双突变体中绒毡层细胞无法形成[21,111]. 此外, *ems1 serk1*双突变体和*ems1 serk1 serk2*三突变体的表型也均类似于*ems1*, 进一步提示了*SERK1/2*和*EMS1*作用于相同的遗传通路来调控绒毡层形成. 后续数据证明, SERK1/2对于EMS1的磷酸化是EMS1发挥其生物学功能的重要条件[112]. 由此, SERK1/2是EMS1的共受体, 且调控绒毡层细胞命运的信号通路被拓展为EMS1-TPD1-SERK1/2.

值得注意的是, 早期研究推测小孢子母细胞可能是细胞分化进程中的一种默认细胞类型, 当绒毡层前体细胞分化受阻时, 其会回归至默认的小孢子母细胞命运, 由此来解释在*ems1*、*tpd1*、*serk1 serk2*突变体中小孢子母细胞增多的表型[15,106]. 后续研究提出, 尽管这些突变体中小孢子母细胞数目增多, 但其药室中已具有部分半分化的绒毡层前体细胞. 据分析, 这些细胞仍具有继续分化及分裂的能力, 且随着更多绒毡层细胞的成功分化, 小孢子母细胞的数目会逐步减少[109]. 因此, 综合上述实验结果, 目前研究认为: 花药形态建成中, 造孢细胞/小孢子母细胞分泌TPD1, TPD1作为EMS1的配体信号分子激活了EMS1-TPD1-SERK1/2信号通路, 该通路确保了绒毡层细胞的正常分化、命运维持和细胞分裂; 与此同时, 绒毡层细胞的形成会反馈限制小孢子母细胞的增殖.

1.2 EMS1-TPD1-SERK1/2信号激活转录因子BES1

基于上述结果, 寻找被EMS1-TPD1-SERK1/2信号途径直接作用的下游因子成为了后续研究的焦点. 油菜素内酯(brassinosteroids, BRs)是一类重要的类固醇激素. 目前, BR作为胞外信号分子, 通过受体激酶BRI1(BRASSINOSTEROID INSENSITIVE 1)和BAK1

表 1　绒毡层发育的相关基因及功能

Table 1　The genes and their functions involved in the tapetum development

功能	基因名	ID	蛋白	定位	文献
绒毡层细胞命运决定	*TPD1*	AT4G24972	分泌短肽	质膜	[19]
	EMS1	AT5G07280	受体激酶	质膜	[20]
	SERK1	AT1G71830	类受体激酶	质膜	[21]
	SERK2	AT1G34210	类受体激酶	质膜	[21]
	BES1	AT1G19350	转录因子	细胞核	[22]
	BZR1	AT1G75080	转录因子	细胞核	[22]
	βCA1	AT3G01500	碳酸酐酶	叶绿体、质膜	[23]
	DYT1	AT4G21330	bHLH转录因子	细胞质、细胞核	[24]
	bHLH010	AT2G31220	bHLH转录因子	细胞核	[25]
	bHLH089	AT1G06170	bHLH转录因子	细胞核	[25]
	bHLH091	AT2G31210	bHLH转录因子	细胞核	[25]
绒毡层细胞特化	*TDF1*	AT3G28470	MYB转录因子	细胞核	[26]
	SKS18	AT1G75790	多铜离子氧化酶	质外体、细胞壁	[27]
	VTC1	AT2G39770	GDP-甘露糖焦磷酸化酶	未知	[28,29]
	EXPB5	AT3G60570	扩展蛋白	细胞壁	[30,31]
	PRX9	AT1G44970	Class III过氧化物酶	未知	[32]
	PRX40	AT4G16270	Class III过氧化物酶	未知	[32]
	GPAT1	AT1G06520	三磷酸甘油酰基转移酶	线粒体	[33]
	GPAT6	AT2G38110	三磷酸甘油酰基转移酶	未知	[34]
	ZmMS33	Zm2G070304	三磷酸甘油酰基转移酶	未知	[35,36]
	AtSEC23A	AT4G01810	COPII蛋白	细胞质、ERES	[37]
	AtSEC23D	AT2G27460	COPII蛋白	细胞质、ERES	[37]
	AtSEC31B	AT3G63460	COPII蛋白	内质网、ERES	[38]
	AtSar1b	AT1G56330	COPII蛋白	ERES、高尔基体	[39]
	ECH	AT1G09330	跨膜蛋白	反式高尔基体	[40,41]
	AP1/2β1	AT4G11380	接头蛋白	反式高尔基体、质膜	[42]
	AP1/2β2	AT4G23460	接头蛋白	反式高尔基体、质膜	[42]
	AP1σ1	At4g35410	接头蛋白	反式高尔基体	[43]
	AP1σ2	At2g17380	接头蛋白	反式高尔基体	[43]
	ISTL1	AT1G34220	ESCRT-III辅助蛋白	未知	[44]
	LIP5	AT4G26750	VPS4/SKD1复合体蛋白	内吞体	[44]
绒毡层细胞程序性死亡	*MON1*	AT2G28390	鸟嘌呤核苷酸置换因子	内吞体	[45,46]
	CEP1	AT5G50260	半胱氨酸蛋白酶	液泡、细胞壁	[47]
	OsAGO2	Os04g0615700	Argonaute蛋白	细胞质、细胞核	[48]
	OsEDM2L	Os08g0502000	腺嘌呤甲基转移酶样蛋白	细胞核	[49]
	RBOHE	AT1G19230	NADPH氧化酶	质膜	[50]
	FAX1	AT3G57280	膜蛋白	质体	[51]
	MADS3	Os10g0201700	MADS-box蛋白	细胞核	[52]
	BnaC.Tic40	AT5G16620	糖蛋白	质体	[53]

(表1续1)

功能	基因名	ID	蛋白	定位	文献
绒毡层参与花粉壁形成相关基因	*ABCG1*	AT2G39350	ABCG转运蛋白	质膜	[54]
	ABCG9	AT4G27420	ABCG转运蛋白	质膜	[55]
	ABCG11	AT1G17840	ABCG转运蛋白	质膜	[56,57]
	ABCG16	AT3G55090	ABCG转运蛋白	质膜	[54]
	ABCG31	AT2G29940	ABCG转运蛋白	质膜	[55]
	ABCG26	AT3G13220	ABCG转运蛋白	质膜	[58,59]
	ACOS5	AT1G62940	脂酰辅酶A合成酶	细胞质、内质网	[60]
	CYP703A2	AT1G01280	细胞色素P450酶	未知	[61,62]
	CYP703B1	AT1G69500	细胞色素P450酶	未知	[63]
	LAP5	AT4G34850	查尔酮合酶	内质网	[64,65]
	LAP6	AT1G02050	查尔酮合酶	内质网	[64,65]
	MS2	AT3G11980	脂酰辅酶A还原酶	质体	[66]
	TKPR1	AT4G35420	多聚α-吡喃酮还原酶	内质网	[67]
	TKPR2	AT1G68540	多聚α-吡喃酮还原酶	细胞质	[67]
	KNS4/UPEX1	AT1G33430	半乳糖基转移酶	未知	[68]
	RES2/QRT3	AT4G20050	聚半乳糖醛酸酶	未知	[69]
	CHS	AT5G13930	查尔酮合酶	细胞核、内质网、液泡膜	[70]
	CHI	AT3G55120	查尔酮黄酮异构酶	细胞核、内质网、液泡膜	[70]
	AMS	AT2G16910	bHLH转录因子	细胞核	[71]
	MS188	AT5G56110	MYB转录因子	细胞核	[72]
	MS1	AT5G22260	PHD-finger转录因子	细胞核	[73]
	ARF17	AT1G77850	生长素响应因子	细胞核	[74]
	TEK	AT2G42940	AT-hook 转录因子	细胞核	[75]
	CIF3	AT5G04030	跨膜蛋白	细胞核、内质网	[76,77]
	CIF4	AT1G28375	跨膜蛋白	细胞核、内质网	[76,77]
	PXL1	AT1G08590	受体激酶	质膜	[78]
	LTP type III	AT5G62080	脂质转运蛋白	内质网、高尔基体	[79]

(BRI1-ASSOCIATED RECEPTOR KINASE 1)共同介导的信号转导通路已被详尽解析[113]. 其中, BES1 (BRI1 EMS SUPPRESSOR 1)和BZR1 (BRASSINAZOLE RESISTANT 1)是BR信号传递通路中的2个关键转录因子[114,115], 通过去磷酸化, BES1/BZR1被激活并在细胞内积累, 进而调控BR下游响应基因的转录. 近期发现, BES1家族的6个成员(BES1、BZR1、BEH1、BEH2、BEH3和BEH4)均在花苞中有表达, 且BES1、BZR1、BEH1和BEH4蛋白集中表达于绒毡层[22,116]. 于是, 研究人员构建多重缺失突变体, 据观察该突变体表型类似于*ems1*、*tpd1*和*serk1 serk2*. *bes1-D*和*bzr1-1D*是BES1和BZR1的功能增强型突变体, 将其分别与*ems1*、*tpd1*、*serk1 serk2*杂交, 能够拯救绒毡层无法正常分化的表型且部分花粉可以发育成熟. 此外, 当TPD1和EMS1存在的情况下, 去磷酸化的BES1能够在细胞核中显著积累[22]. 这些结果表明: BES1家族成员可以通过非BR依赖的方式同时受到EMS1-TPD1-SERK1/2信号途径的激活, 从而促使绒毡层细胞的形成.

另一方面, 有研究指出: BES1还可以直接结合到控制绒毡层发育(*DYT1*、*TDF1*、*MS188*和*MS1*)和花粉壁合成的一系列基因的启动子区域[22,117], 这提示了BES1不仅参与调控绒毡层细胞的形成, 其可能对后续绒毡层细胞的特化、降解和花粉壁建成也起到重要

作用. 综上, 这些研究极大地拓展了EMS1-TPD1-SERK1/2信号通路调控绒毡层形成和发育的分子机制.

1.3　β-碳酸酐酶——EMS1直接作用的信号分子

碳酸酐酶(carbonic anhydrases, CAs)是一种锌金属酶, 广泛存在于动物、植物、微生物及藻类中, 高效催化CO_2和HCO_3^-之间的转换反应[118]. 在动物中, CAs主要涉及脂质新生、尿素合成、毒力调节和肿瘤形成等病理学过程[119]. 在植物中, 根据系统进化将其分为α、β和γ三类, 其中βCAs家族占主导地位[120,121]. 拟南芥βCAs有6个成员(βCA1~βCA6), 研究发现, EMS1受体激酶能与βCA1、βCA2、βCA4体内互作. 据分析, 这3个蛋白均在花药发育中表达, 且βCA1更集中在第5~6期绒毡层细胞膜和细胞质中定位, 提示了βCA1, βCA2和βCA4可能是EMS1作用的下游蛋白, 参与绒毡层细胞的发育过程[23].

进一步研究表明, *βca1 βca2 βca4*突变体中出现了半分化的绒毡层类似细胞, 但由于其后续无法正常发育, 导致花粉完全败育; 相反, 过量表达*βCA1*会诱导产生更多的绒毡层细胞, 亦会干扰花药形态建成, 导致育性下降. 生化证据显示, EMS1能够在不依赖TPD1的情况下实现对βCA1的磷酸化, 以此提高其碳酸酐酶的催化活性. 另一方面, 两个关键的遗传学实验证实EMS1通过磷酸化βCA1来调控绒毡层细胞的分化: (1) βCA1的关键磷酸化位点被突变后无法拯救*βca1 βca2 βca4*的表型; (2) 编辑磷酸化位点增强βCA1酶活性会造成绒毡层细胞数目的紊乱[23]. 综上研究表明: 除了EMS1-TPD1-SERK1/2信号通路激活BES1及其他成员外, EMS1还能作用于βCAs信号分子, 由此实现不同分子通路对绒毡层细胞形成和发育的精准调控.

2　绒毡层细胞特化

绒毡层细胞形成后, 随即通过细胞特化(第二次分化)成为分泌型细胞. 分泌型绒毡层细胞的主要特征为[122]: (1) 细胞内形成两个或多个细胞核(双/多核化); (2) 细胞液泡化, 细胞膨大, 细胞壁逐步降解; (3) 细胞质浓密、内膜系统细胞器丰富(图1). 由此, 特化后的分泌型绒毡层具备了大量合成、储存以及极性运输物质的能力, 而这些均是满足小孢子/花粉顺利发育的细胞学基础. 显然, 绒毡层特化所经历的这一系列生物学过程涉及到复杂的分子调控网络, 但是, 目前对于调控绒毡层特化的分子机制研究仍十分有限.

2.1　绒毡层由细胞分裂转向细胞特化的调控通路

已报道, 拟南芥R2R3 MYB家族转录因子TDF1对于绒毡层早期发育起到重要的调控作用. *tdf1*突变体中, 绒毡层细胞虽已形成但其细胞分裂异常、部分区域形成非单一细胞层, 绒毡层细胞无法特化, 后期细胞大量空泡化, 最终呈现完全雄性不育[26,27].

后续研究发现, TDF1直接激活下游基因*SKS18* (*SKEWED5-SIMILAR 18*)表达, 该蛋白在第6-7期绒毡层细胞的质外体和细胞壁上定位[27]. SKS蛋白属于多铜离子氧化酶家族(multicopper oxidase-like proteins, MCOs). 在高等植物中, 抗坏血酸氧化酶是MCOs的一大亚类[123], 其可对还原型抗坏血酸(维生素C)进行氧化[124,125]. 据报道, 抗坏血酸对细胞增殖起到调控作用[126~128]. 于是, 通过体外酶活实验, 研究人员证实SKS18以铜离子作为辅因子发挥抗坏血酸氧化酶活性[27]. *sks18*突变体表型显示: 尽管育性未受到影响, 但绒毡层细胞数目异常增加; 而过量表达*SKS18*可以抑制*tdf1*中绒毡层细胞的分裂, 甚至部分恢复绒毡层细胞的特化进程. 另一方面, VTC1 (VITAMIN C DEFECTIVE1)是抗坏血酸合成途径中的限速酶[28,29], 该蛋白在绒毡层中的积累受到TDF1的负向调控; 利用特异启动子过量表达*VTC1*于野生型背景中也会导致绒毡层细胞的多分裂[27]. 以上结果证明, TDF1-SKS18分子模块促进抗坏血酸的氧化, 同时, TDF1-VTC1分子模块抑制抗坏血酸的合成; 由此, TDF1作为抗坏血酸积累的负控因子作用于绒毡层由细胞分裂转向细胞特化.

抗坏血酸是植物体内主要的抗氧化剂, 通过抗坏血酸-谷胱甘肽循环, 对清除活性氧(reactive oxygen species, ROS)起到核心作用[129]. 在此基础上, 利用特异染料对绒毡层中的ROS积累水平进行检测, 结果显示: 相较于野生型, 绒毡层ROS含量在抗坏血酸异常积累的遗传材料中均呈下降趋势. 遗传学分析进一步证实, ROS积累不足亦会使绒毡层持续异常的细胞分裂、阻碍细胞特化[27]. 综上所述, 该研究揭示TDF1通过调控抗坏血酸代谢途径影响ROS内稳态, 从而促使绒毡层由细胞分裂至细胞特化的分子机制.

2.2 绒毡层细胞壁的完整性

植物细胞壁的结构成分主要为纤维素、半纤维素、果胶多糖等, 通过共价键和非共价键的连接形成一个坚实的网络结构. 因此, 完整的细胞壁既具有弹性也保持强度, 这赋予了其控制细胞体积、决定细胞形态和保护原生质体的能力. 花药发育中, 绒毡层细胞壁也兼顾伸展性和坚固性, 由此充分匹配花粉发育各阶段的变化.

随着小孢子母细胞进行减数分裂, 绒毡层细胞内许多小液泡融合成一个中央大液泡(图1). 由于液泡体积的增长会先发生于细胞体积的增加, 进而引起原生质体挤压细胞壁, 细胞膨压上升. 大量实验证据表明, 膨压会促使细胞壁松弛, 细胞壁通过其扩张来增大细胞体积, 从而降低细胞的膨压、减小胞壁承受的压力. 据报道, 扩展蛋白(expansins)能够削弱壁多糖之间的非共价键促使胞壁延展[130~132]. 根据序列比对的相似性, 扩展蛋白主要分为α和β两大类; 拟南芥中有26个α-expansin基因和5个β-expansin基因[133]. *EXPB5*基因编码一个β-expansin蛋白. 花药发育第6~7期, *EXPB5*在绒毡层细胞中有较高表达, 而在*tdf1*突变体中其表达特异下调. 体内与体外实验证明, *EXPB5*基因的转录激活依赖于TDF1-AMS复合体的调控[30]. 因此, 推测TDF1-EXPB5分子模块可能直接作用于绒毡层细胞壁, 通过选择性疏松细胞壁使细胞膨大, 由此来减轻绒毡层特化过程中的物理压力. 近期, 大麦中的研究指出: 直系同源基因*HvTDF1*可以功能互补拟南芥*tdf1*突变体表型; 且*HvTDF1*能够调控3个渗调蛋白(osmotins)的表达[85]. 渗调蛋白是一类逆境适应蛋白, 主要集中在液泡, 目前推测该类蛋白参与渗透调节、维持细胞膨压和传递胁迫信号等[134,135]. 该团队认为, HvTDF1通过启动渗调蛋白对细胞溶质浓度或是水势变化进行调整, 其结果有可能反馈作用于β-expansin蛋白, 最终依托细胞壁扩张实现对绒毡层细胞的物理保护和体积调控[85].

另一项研究指出, 拟南芥Class III过氧化物酶PRX9 (PEROXIDASE9)和PRX40 (PEROXIDASE40)也对维持绒毡层细胞壁的完整性起到重要作用[32]. *PRX9*和*PRX40*在第6~9期绒毡层中特异表达. 表型分析显示, *prx9*和*prx40*单突变体均正常发育; 而*prx9 prx40*双突变体中绒毡层细胞肿胀, 细胞壁会嵌入至相邻细胞中, 无法保持正常形态; 花粉外壁的沉积模式也受到干扰, 最终导致小孢子完全降解. 早期研究发现, 拟南芥Class III过氧化物酶家族共有73个成员[136,137], 一些成员被认为参与聚合植物细胞壁的部分成分包括木栓质(suberin)和伸展蛋白(extensin)[138,139]; 相反, 另一些成员可能通过产生羟基自由基来切割细胞壁多糖、打破胞壁的稳定性[140]. 生化证据表明, PRX9和PRX40通过酪氨酸残基对伸展蛋白进行分子内或分子间的交联[32]. 伸展蛋白是细胞壁中富含羟脯氨酸的糖蛋白, 控制着细胞的大小和形态[141,142]. 结合上述结果, PRX9和PRX40作为过氧化物酶可能通过交联伸展蛋白起到支撑绒毡层细胞壁的作用, 从而保证绒毡层细胞壁在扩张的同时不丧失其既有结构的稳定性.

2.3 绒毡层内膜系统细胞器和分泌途径

绒毡层细胞特化中拥有丰富的内膜系统细胞器, 包括内质网、高尔基体、反式高尔基体/早期内吞体(trans Golgi network/early endosome, TGN/EE)、液泡和质膜等[143]. 除此之外, 绒毡层细胞还分化出其特有的细胞器, 包括由内质网发育而来的绒毡层小体(tapetosome)和由质体发育而来的造油体(elaioplasts)[144,145]. 通过这些细胞器大量的内容物被合成及加工, 并由转运囊泡定向的运输至目标部位[146,147], 执行生物学功能. 花粉壁形成的研究中发现, 组成花粉壁的前体物质一部分会通过特定的质膜转运蛋白(表1)或是分泌途径由绒毡层转运至小孢子表面; 另一部分则会存储于绒毡层小体和造油体中, 随着绒毡层PCD定位至花粉壁表面. 因此, 花粉壁形成极大程度地依赖于绒毡层细胞发达的内膜系统, 下文也将重点介绍绒毡层各细胞器介导的内膜运输对于花粉壁发育的分子调控.

内质网是由小管连接的多边形网络和潴泡形成的平面囊构成. 拟南芥甘油-3-磷酸酰基转移酶(glycerol-3-phosphate acyltransferase, GPAT)是催化甘油脂合成途径的关键酶, 对于膜脂质、贮存脂质和细胞外脂质聚酯的生物合成发挥重要作用[148]. GPATs共有10个成员, 其中*AtGPAT1*、*AtGPAT4*和*AtGPAT6*在花苞中富集. 针对*AtGPAT1*基因功能的研究发现: 从第8期开始, *atgpat1*突变体绒毡层细胞肿胀且后续显示出PCD延迟, 最终导致育性下降. 透射电子显微镜结果表明, *atgpat1*内质网潴泡明显减少, 完整的线粒体结构缺失. 此外, 突变体药室内围绕花粉的囊泡大量减少, 提示*AtG-*

*PAT1*的功能缺失会影响绒毡层的分泌能力. 生化证据显示, AtGPAT1可特异地对甘油-3-磷酸进行催化, 对花序中的脂肪酸进行修饰[33]. 另一项研究发现, *atgpat6*突变体绒毡层细胞也类似于*atgpat1*; 其花粉外壁结构不完整. *atgpat1 atgpat6* 双突变体则呈现完全雄性不育表型; 绒毡层细胞在第7期异常膨大、细胞严重空泡化. 利用特异性荧光染料苯胺蓝分析胼胝质降解情况: 第8期, 野生型、*atgpat1*和*atgpat6*花药中无法观察到荧光信号, 说明四分体胼胝质被顺利降解, 小孢子已被释放入药室腔中; 而*atgpat1 atgpat6*荧光信号滞留, 未观察到小孢子, 提示绒毡层可能无法正常分泌胼胝质酶降解胼胝质[34]. 综上, 这些研究推测*AtGPAT1*和*AtGPAT6*主要通过脂生物合成途径影响绒毡层内质网的发育, 继而作用于绒毡层分泌和花粉壁形成.

大量货物由内质网合成后, 会先被COPII包被蛋白(coat protein II)包裹, 形成的COPII包被囊泡转运至高尔基体[149]. 目前, 多项研究发现, 拟南芥COPII复合体对绒毡层特化以及花粉壁形成起到关键作用. COPII复合体由5个蛋白质组成, 分别为Sar1(Ras-related protein), SEC23, SEC24, SEC13和SEC31[150]; 拟南芥COPII复合体高度保守, 但存在更多的蛋白亚型[151], 其在绒毡层/花粉壁发育中的共同点为: (1) *AtSEC23A/D*, *AtSEC31B*, *AtSar1b*在特化阶段的绒毡层细胞中高表达[37~39]; (2) 亚细胞定位显示AtSEC23A/D, AtSEC24A/B/C, AtSEC31B, AtSar1a蛋白位于COPII复合体聚集的内质网退出位点(ERESs)[37,38,152,153]; (3) *atsec31b*, *atsar1b*单突变体和*atsec23ad*双突变体中, 造油体内的质体小球减少、绒毡层小体内部结构不清晰; 部分孢粉素错误的向中层方向转运; 花粉外壁沉积混乱[37~39]; (4) 异位表达AtSEC31A可以拯救*atsec31b*表型[154], 同样地, AtSar1c可以拯救*atsar1b*表型[39], 提示AtSEC31A/B, AtSar1b/c两两的功能存在可替换性. 当然, 上述突变体也存在表型上的差异性, 例如: *atsec31b*四分体内指导孢粉素沉积的初生外壁基质特异减少[38]; *atsar1b*则可能因低效转运半胱氨酸蛋白酶导致绒毡层PCD延迟[39], 由此提示COPII复合体各组分对特定货物转运的选择性. 综上结果, 推测COPII复合体通过内质网-高尔基体途径参与不同物质在绒毡层胞内/外的极性转运, 从而有序地调配绒毡层的分泌进程.

植物特有的TGN/EE是细胞的分选中心, 通过接头蛋白(adaptor protein, AP)和网格蛋白(clathrin)形成的网格蛋白包被囊泡(clathrin-coated vesicles, CCVs)继续介导货物由TGN/EE转运至质膜或是胞外, 完成胞吐途径[149]. 在此途径中, 研究已证明跨膜蛋白ECH (ECHIDNA)定位于TGN/EE, 它能促使TGN形成分泌囊泡, 将蛋白质包括BRI1、AUX1 (AUXIN RESISTANT 1)或是细胞壁成分转运至细胞膜[40,155,156]. 花药发育中, ECH缺失导致育性严重下降[41]. 结合突变体表型和组织化学染色等实验, 该作者提出定位于绒毡层的ECH可能通过TGN/EE网络运输细胞壁组分, 一方面提供花粉壁的结构成分、实现花粉壁对小孢子的物理保护; 另一方面, 促使后期内皮层次生壁加厚、辅助花药开裂.

CCVs的生成起始于接头蛋白AP复合体招募网格蛋白、辅助蛋白和货物蛋白. AP复合体分为AP-1~AP-5, 其中AP1γ、AP1/2β、AP1μ和AP1σ四个亚基组成AP-1复合体; AP2α、AP1/2 β、AP2μ和AP2σ组成AP-2复合体[157]. 近期, 国内外团队揭示了拟南芥绒毡层细胞中不同AP复合体亚基介导的蛋白胞吐途径调控花粉壁发育, 具体为: (1) AP-1和AP-2共有的β1β2亚基定位于绒毡层细胞的TGN/EE, β1β2能够与ABCG9(ATP-binding cassette G9)、ABCG16(ATP-binding cassette G16)跨膜蛋白(表1)互作, 通过TGN/EE囊泡转运途径将ABCG9/16靶向定位至细胞膜, 为后续花粉壁前体物质分泌至小孢子提供必要的转运途径[42]. (2) 脂酰辅酶A合成酶(acyl-CoA synthetase 5, ACOS5)对孢粉素单体进行修饰[60], type III 脂质转运蛋白(lipid transfer proteins, LTP)则可能转运脂质至花粉壁[79]. 据报道, AP-1σ亚基对这两个绒毡层可溶性蛋白向药室腔的分泌起到调控作用[43]. 值得注意的是, AP-1σ亚基也会影响造油体和绒毡层小体的发育, 提示AP-1复合体介导的网格蛋白囊泡运输对绒毡层内膜细胞器的发育也发挥作用.

植物内膜运输中, 内体蛋白分选转运装置(endosomal sorting complexes required for transport, ESCRT)识别被泛素化修饰的货物, 然后将这些货物分选到多囊泡体/液泡前体(multivesicular bodies/ prevacuolar compartents, MVBs/ PVCs)的腔内囊泡中, 接着成熟的多囊泡体与液泡融合, 货物最终被液泡中的水解酶降解[158]. 显然, 多囊泡体介导的运输途径参与物质降解, 但相关研究证实其也可作为一种非经典的分泌途径参

与物质外运，即对经典的分泌途径进行有效地补充[159]. 拟南芥ESCRT超级复合物参与多囊泡体的形成，主要由ESCRT-I、ESCRT-II、ESCRT-III和VPS4 (vacuolar protein sorting-associated protein 4)组成，其中ISTL1 (IST1-LIKE1)和LIP5 (LYST-INTERACTING PROTEIN 5)是两个辅助蛋白[160]. 据报道，ISTL1和LIP5功能的同时缺失不仅影响绒毡层细胞中TGN形态，还会导致多囊泡体显著变小；内部的腔内囊泡减少、体积异常增大，表明ISTL1和LIP5对多囊泡体的发生起到重要作用. 研究进一步表明，ISTL1和LIP5能协同运输ABCG9、type III LTP至绒毡层细胞膜，提示了ESCRT介导的多囊泡体途径参与花粉壁形成相关蛋白的转运和分泌[44].

3 绒毡层细胞程序性死亡

多细胞生物体中，由基因调控产生的细胞主动死亡过程称为程序性死亡. 花药发育中，绒毡层细胞也经历PCD，该过程始于第10期，终于第12期. 大量研究表明，PCD不仅为花粉提供了必要的发育空间；同时，是释放造油体、绒毡层小体等绒毡层内容物的前提. 此外，绒毡层PCD的发生时序也十分关键，提前或延迟绒毡层PCD均会导致花粉败育. 目前，多项研究表明水解酶、ROS内稳态、表观遗传修等涉及到绒毡层PCD的分子调控机制.

3.1 半胱氨酸蛋白酶促进绒毡层PCD

早期证据表明，绒毡层PCD开启依赖于半胱氨酸蛋白酶CEP1 (CYSTEINE ENDOPEPTIDASE 1)[47]. 花药发育第6~10期，*CEP1*特异在绒毡层细胞中表达. 细胞学观察发现：当*CEP1*缺失时，绒毡层PCD延迟，其内容物无法正确分泌至小孢子表面，造成花粉外壁形成受阻；而当*CEP1*过量表达时，绒毡层PCD提前，最终小孢子细胞质降解、其表面堆积着过量且无序的孢粉素前体[47]. 此外，对野生型、功能互补和过表达转基因材料进行比较，发现CEP1蛋白水平超过野生型阈值后，花粉出现败育且败育程度与CEP1表达呈正相关性[47]. 因此，CEP1通过调控绒毡层PCD促进花粉正常发育.

进一步分析CEP1功能，发现该蛋白首先以蛋白酶原的形式出现于多囊泡体中，经转运至液泡内才能转化为有活性的成熟酶[47]. 后续研究表明，拟南芥鸟嘌呤核苷酸置换因子MON1 (MONENSIN SENSITIVITY1)/CCZ1 (CALCIUM CAFFEINE ZINC SENSITIVITY1)能够激活Rab7(Rab GTPase)，通过促使多囊泡体与液泡的融合将介导绒毡层PCD的半胱氨酸蛋白酶转运至液泡. 综合以上结果，推测CEP1可能通过MON1-Rab7介导的囊泡转运途径定位于液泡从而启动绒毡层PCD. 最终，随着绒毡层细胞的彻底降解(图1)，黄酮、链烷类、固醇酯类和含油层等物质被释放至花粉外壁，完成花粉壁建成[45,46].

3.2 ROS内稳态调控绒毡层PCD

ROS作为重要的信号分子，其在拟南芥和水稻花药中的时空分布呈现出相似性. 具体来说，ROS在野生型花药第6~7期逐步升高，第8~9期达到高峰，之后逐渐回落，直到第12期再次上升[50,52]. 后续研究发现，ROS对于调控绒毡层PCD的正确发生起到重要作用. 拟南芥*RBOHE* (*RESPIRATORY-BURST OXIDASE HOMOLOG*)编码NADPH氧化酶，其定位于质膜上并主要产生O_2^-,而大部分O_2^-会转化为H_2O_2积累于质外体中. 花药发育中，*RBOHE*在第6~11期的绒毡层细胞中特异表达[50]. 表型分析显示，*rbohe*突变体中绒毡层PCD延迟；相应的，利用绒毡层特异启动子过量表达*RBOHE*于野生型背景会导致绒毡层提前PCD. TUNEL实验表明，相较于野生型绒毡层中第10期出现的PCD信号，突变体中该信号在第11期出现，而在*RBOHE*过表达转基因材料中该信号提前至第9期. 进一步利用特异染料检测花药中ROS积累水平，结果显示：第6~11期*rbohe*花药中ROS含量对比野生型显著下降；而在过表达转基因株系中ROS含量由第6期开始持续维持较高水平[50]. 由此可见，RBOHE通过产生ROS来促进绒毡层PCD，且绒毡层PCD的时序性主要依赖于ROS的时空表达模式.

水稻中，*OsMADS3*编码C-class MADS box转录因子，主要在第9~12期绒毡层细胞中表达[52]. 转录组学和生化数据证明，OsMADS3能够直接结合于下游基因*MT-1-4b*的启动子区域. *MT-1-4b*编码一个金属硫因蛋白，酶活实验表明MT-1-4b具有清除ROS的催化活性. 利用细胞学和组织染色观察发现，*MADS3*和*MT-1-4b*基因敲低突变体中绒毡层细胞提前降解且花药中ROS含量相较于野生型均显著增加[52]. 因此，推测Os-

MADS3通过激活*MT-1-4b*表达实现对ROS的清除，由此平衡ROS内稳态、保证绒毡层PCD的正常进行. 值得注意的是，抗坏血酸氧化酶SKS18能够通过氧化抗坏血酸促使ROS积累于绒毡层[27]，提示了抗坏血酸也可能作为ROS的调节因子作用于绒毡层PCD过程[161]. 综上, ROS内稳态是调控绒毡层PCD的重要途径.

3.3　表观遗传修饰调控绒毡层PCD

表观遗传因子(包括DNA甲基化, 组蛋白修饰和非编码RNA)对于植物生殖发育起到关键的调控作用. 近期，两项关于水稻花药发育的研究进一步揭示了表观遗传修饰调控绒毡层PCD的分子机制. 水稻中有19种预测的AGO (ARGONAUTE)蛋白，推测其可能参与RNA介导的基因沉默. 该项研究表明，OsAGO2在绒毡层细胞中高度表达且影响花粉形成[48]. 分析*ago2*突变体，发现绒毡层细胞中ROS含量过度积累导致PCD提前、花粉败育. 进一步研究表明, OsAGO2能够直接结合在*OsHXK1* (*HEXOKINASE 1*)启动子区域, 通过调节DNA甲基化水平影响*OsHXK1*表达. OsHXK1被认为参与ROS产生. 于是，构建*OsHXK1*过表达材料并发现其表型类似于*ago2*突变植株. 此外, 遗传学证据表明在*ago2*突变植株中敲低*OsHXK1*能够拯救部分花粉、恢复育性[48]. 以上结果表明，OsAGO2直接对*OsHXK1*进行甲基化修饰，通过抑制*OsHXK1*表达来调节ROS内稳态从而控制绒毡层PCD的发生时间.

早期研究证明，转录因子bHLH142能够与TDR (TAPETUM DEGENERATION RETARDATION)互作激活*EAT1* (*ETERNAL TAPETUM 1*) 表达; EAT1则继续启动*OsAP25*和*OsAP37*转录，通过表达这两个天冬氨酸蛋白酶促进绒毡层PCD[162]. 近期研究发现，OsEDM2L (ENHANCED DOWNY MILDEW 2-LIKE)作为一个水稻EDM2类似因子，特异在绒毡层中表达; 其功能缺失突变体中绒毡层PCD延迟、花粉败育[49]. 生化数据表明，OsEDM2L能够与 bHLH142、TDR互作调控*EAT1* 表达. 另一方面，转录水平m^6A定量结果显示: *osedm2l*突变体中，*EAT1*转录本的m^6A修饰模式较野生型发生显著变化，这导致*EAT1*转录本的可变剪接与加尾表现异常，由此阻碍了下游天冬氨酸蛋白酶的表达、造成绒毡层降解延迟[49]. 因此，该研究揭示了一个植物特有的m^6A表观调控因子作用于绒毡层PCD的分子机制.

3.4　质体发育与绒毡层PCD

绒毡层细胞中的质体经发育形成造油体，储存甾醇酯等物质. 多项研究已发现，绒毡层质体发育异常往往伴随着PCD延缓表型[53,163]. 近期研究指出，*OsCPPR1* (CYTOPLASM-LOCALIZED PPR1)编码一个含有16个PPR基序的P型PPR蛋白，定位于细胞质中[164]. 花药发育中，*OsCPPR1*主要在第10期绒毡层细胞中高表达，提示*OsCPPR1*可能参与在花药发育后期绒毡层PCD过程. 表型分析显示，*cppr1*突变体中绒毡层细胞内的质体发育异常、绒毡层降解延迟. 为了进一步解析*OsCPPR1*调控绒毡层发育的分子机制，研究团队分析转录组数据，发现转录因子OsGLK1 (OsGOLDEN-LIKE1)在*cppr1*突变体中表达显著上调. 已有研究报道表明OsGLK1在质体发育中起关键作用. 于是，利用RIP、RNA-EMSA、RNA稳定性测定等结果证实OsCPPR1直接结合至*OsGLK1* mRNA单链区域，可能通过剪切和降解*OsGLK1* mRNA来下调其转录水平. 遗传学证据也表明，*OsGLK1*过表达材料中绒毡层质体未能分化成正常的造油体、绒毡层细胞自身异常膨大、PCD延缓, 该些表型类似于*cppr1*突变植株; 而在*cppr1*突变植株中抑制*OsGLK1*表达能够部分恢复花粉育性[164]. 综上，该研究揭示OsCPPR1通过抑制*OsGLK1*表达调控绒毡层质体发育以及PCD，而对于绒毡层质体发育和PCD的内在联系在今后仍值得继续深入探索.

4　绒毡层与花药各层的协同合作

随着花药发育研究的深入，相关报道逐步揭示了绒毡层与中层、与内皮层以及小孢子之间的分子联系. 凭借这些分子调控途径, 花药各层协同互作, 最终保证花药发育各环节能够同步匹配、为成熟花粉的顺利产生奠定基础.

4.1　CIF-GSO信号转导途径作用于绒毡层、中层和小孢子

拟南芥GSO1/2 (GASSHO)跨膜受体激酶共有5个配体，分别为TWS1 (TWISTED SEED1)和CIF1-CIF4 (CASPARIAN STRIP INTEGRITY FACTOR). 已报道, CIF-GSO信号转导途径参与调控胚胎角质层和凯氏带

形成过程[76,165,166]. 针对花药发育的研究[77], 发现GSO1/2从第6期开始特异定位于中层, CIF3/4则定位于绒毡层. 表型分析显示, *gso1*和*gso2*单突变体均能够正常发育, 而*gso1 gso2*双突变体中绒毡层细胞异常膨大; 部分孢粉素被错误地朝中层方向分泌; 成熟花粉粒互相黏连成团. 此外, 相似的表型也出现于*cif3 cif4*双突变花药中. 前期生化数据证明, TWS1多肽前体需要经C端枯草杆菌蛋白酶(C-terminal subtilase, SBT)催化后才具有活性[76]. 因此, 研究人员进一步筛选了在花药中表达的SBT, 发现SBT5.4蛋白能够通过切除CIF4多肽前体的部分C末端激活活性. 有趣的是, 蛋白定位显示SBT5.4特异在第8期后的小孢子中表达. 以上结果表明, CIF-GSO信号转导途径涉及到绒毡层、小孢子和中层之间的协同互作: 绒毡层细胞分泌CIF3/4多肽前体至药室腔; 接着, 这些多肽前体被小孢子中的SBT5.4激活; 产生的活性CIF3/4配体弥散至中层, 通过结合GSO1/2受体启动CIF-GSO信号转导途径, 最终作用于绒毡层和花粉壁的发育.

尽管目前对于CIF-GSO信号转导途径作用的下游因子仍不清楚, 但多个遗传学证据表明该信号通路中受体与活性配体的时空表达对于花粉外壁模式决定十分重要[77]. 故该作者推测, GSO于中层的定位可能是避免干扰绒毡层细胞内其他信号途径的一种策略; 亦或是这种由中层细胞定向释放出的下游信号会确保绒毡层细胞的极性分泌. 综上, 该研究揭示了绒毡层、中层和小孢子三者之间协同合作的作用模式.

4.2 PXL1-CLE19-SERKs信号转导途径作用于绒毡层和小孢子

拟南芥中, CLAVATA3/EMBRYO SURROUNDING REGION-RELATED多肽配体家族至少存在32个成员[167]. 其中, *CLE19* (*EMBRYO SURROUNDING REGION-RELATED 19*)、*CLE9*、*CLE16*、*CLE17*、*CLE41*和*CLE42*在花药中表达, 提示这些成员在花药发育中可能存在功能冗余[168]. 之后, 细胞学、转录组学和遗传学证据表明, 在小孢子中表达的CLE19及其同源多肽可作为“刹车”信号来抑制绒毡层花粉壁调控因子AMS及其下游的转录级联网络, 精确地调控花粉壁前体物质的合成与积累, 维持花粉壁的正常形态. 然而, CLE19与哪些跨膜受体组成信号通路仍不清楚.

近期, 上述科研团队继续探索[78], 发现 CLE19直接与PXL1 (PXY-LIKE1)跨膜受体激酶的胞外LRR结构域互作, 诱导PXL1磷酸化. *PXL1*基因在绒毡层细胞中表达, 其蛋白缺失突变体中花粉外壁前体物质过多沉积、干扰了花粉壁的蜂窝状结构模式, 该表型类似于*cle19*, 证明PXL1作为CLE19受体在花粉壁发育中起作用. 此外, PXL1、PXL2 (PXY-LIKE2)和PXY (PHLOEM INTERCALATED WITH XYLEM)在序列上高度相似; 表型分析显示, *pxl1 pxl2 pxy*三突变体表型比*pxl1*更为严重, 提示这三者在花粉壁发育过程中存在功能冗余. SERKs是多个RLK信号途径中的共受体, 例如上述EMS1-TPD1-SERK1/2信号转导途径. 进一步生化数据证实, CLE19可以诱导PXL1-SERK1/2/3形成复合体及交互磷酸化, 所以SERKs也可作为PXL1共受体参与介导胞内信号的转导. 因此, 结合前期结果, 该项研究阐明小孢子通过PXL1-CLE19-SERKs信号转导途径调控绒毡层转录通路的分子机制, 揭示了绒毡层和小孢子协同保障花粉壁发育的作用模式.

4.3 ZmMs33/ZmGPAT6作用于绒毡层和内皮层

前期基因克隆和功能验证表明, 玉米*ZmMs33*编码甘油-3-磷酸酰基转移酶(GPAT), 参与调控绒毡层和花粉壁的发育过程[35]. 近期, 该团队系统探究了*ZmMs33*调控雄性不育的分子机理[36], 揭示了不同于拟南芥GPATs的生物学途径. 研究发现, 野生型花药中, 内皮层叶绿体在花药发育早期承担了白天合成、存储淀粉粒, 夜晚降解淀粉粒、实现物质代谢的生物学功能; 而在花药发育后期, 叶绿体直接参与光合作用, 为花药发育提供能量. 透射电子显微镜结果表明, *ms33*突变体内皮层的叶绿体结构异常, 因此丧失了淀粉周转和光合作用的功能, 提示ZmMs33参与维持内皮层细胞中叶绿体的正常发育. 原位杂交显示, *ZmMs33*主要表达于花药发育早期的绒毡层细胞. 生化数据进一步证实, ZmMs33是甘油脂合成途径的第一步; 相应的, 靶向代谢组学显示ZmMs33的功能缺失会抑制糖脂和磷脂的生物合成. 脂类是叶绿体膜形成的主要成分, 因此, 绒毡层ZmMs33参与的脂代谢及其产物是确保内皮层叶绿体正常发育的重要条件.

众所周知, 叶绿体是供给碳源和能量的内膜细胞器. 因此, 研究人员检测了*ms33*花药中的18种主要代谢物, 通过比较野生型, 发现突变体中的葡萄糖、果糖等糖类物质含量紊乱、ATP含量显著降低, 提示了

内皮层叶绿体是输送花药碳源和能量的关键途径之一. 据报道, 当植物处于低能量或是“饥饿”状态下, “能量感受器”“自噬诱导因子”SnRK1 (sucrose non–fermenting-related kinase 1)蛋白复合体会被激活, 从而促进自噬并对代谢过程进行重排[169]. 实验表明, *ms33*突变体中, *SnRK1β*和*SnRK1γ*调控亚基的基因表达上升; SnRK1α催化亚基的磷酸化激活态也显著积累. 相对应的, 细胞学显示绒毡层中出现自噬液泡和过早PCD; 花药内部也因为处于低能量和低物质状态停止发育, 最终导致完全败育. 综上, 该研究阐明了绒毡层关键酶ZmMs33促进内皮层叶绿体发育的分子机制, 揭示出绒毡层和内皮层通过协同互作调节花药正常发育的作用模式.

5　总结与展望

综上可见, 对于绒毡层发育的研究已经跨越了1个多世纪, 国内外科研团队也通过不同的角度逐步地在完善绒毡层发育的分子网络. 凭借这些基础理论, 近年来中国作物育性的研究和应用也得到了拓展[170~176], 这为丰富雄性不育系种质资源、优化杂交制种技术体系提供了遗传改良的方向.

尽管如此, 绒毡层发育中仍有许多生物学过程值得被深入解析: (1) 绒毡层细胞双/多核化是细胞特化的重要标志[177]. 然而, 对于双/多核化的生物发生机制及细胞器功能的理解十分有限. 有趣的是, 动物细胞中也存在双核化包括心肌细胞、肝实质细胞、乳腺细胞等[178]. 目前, 对于这些细胞为何出现、如何产生双核也不明确, 虽然逻辑上推测双/多核化带来的基因组倍性增加可能是促进细胞分化、细胞扩张或是适应不利环境的特殊机制[179]. (2) 大部分分泌型绒毡层细胞壁的降解是逐步完成的. 具体来说, 靠近小孢子方向的绒毡层细胞壁会先降解; 相邻细胞间辐射壁的降解紧随其后, 最终胞间连丝会在花药发育后期消失[180]. 但是, 目前对于绒毡层细胞壁降解的分子调控途径仍是空白. (3) 植物激素参与众多生理和生化过程. 已有报道表明, 生长素、BR、乙烯等在调控花药/花粉发育中起到关键作用[181~184], 但显然, 各植物激素作为内源信号对绒毡层发育的分子调控及其分子网络间的联系仍亟待研究.

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Advance in the anther tapetum development and their regulatory mechanisms

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During the male reproductive development in plants, the pollen grains (male gametophytes) production is the basis for pollination, fertilization and seed setting. In the stamen of higher plants, the pollen grains are formed in anthers. The anthers consist of the multiple cell layers. The tapetum is the innermost layer of the anther walls, directly providing necessary nutrients, raw materials and energy for microspores and/or developing pollen grains. Conversely, the defective tapetum leads to the pollen abortion and male sterility. In agricultural production, the male sterility is an important agronomic trait, also the basis for hybrid breeding. Therefore, study the molecular mechanisms of tapetum development not only expand the understanding of male reproductive development in plants, but also provide the theory for guiding the new genetic resources creation and improving the crop breeding design capacity. During the anther development, the tapetal cells undergo three main stages: cell identify, cell re-differentiation and programmed cell death. Combining with the recent researches, in this review, we focus on the key biological events, introduce the gene functions and molecular pathways involved in the tapetal developmental stages, systematic elaborating the molecular basis underlying the tapetum development associated with other anther walls coordination.

tapetum, cell identify, cell re-differentiation, pollen wall formation, anther wall, male sterility

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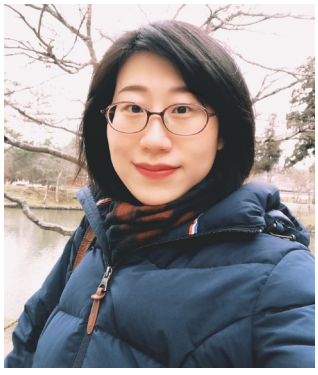

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