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·综 述·

生物合成薯蓣皂素的途径设计及关键酶分析

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摘 要: 薯蓣皂素是一种天然甾体皂苷元，可作为数百种类固醇药物的前体，具有重要药用价值。目前工业生产薯蓣皂素主要依赖化学提取法，因此该法依赖植物材料和耕地且对环境有害。随着代谢工程和合成生物学的发展，生物合成法受到广泛关注。文中综述了生物合成薯蓣皂素的代谢途径和关键酶，并在酿酒酵母 *Saccharomyces cerevisiae* 中设计其异源合成途径，提出改造策略，以期为全生物合成薯蓣皂素提供有价值的参考。

关键词: 薯蓣皂素，生物合成途径，关键酶，异源表达

Pathway design and key enzyme analysis of diosgenin biosynthesis

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Abstract: As a naturally occurring steroid sapogenin, diosgenin acts as the precursor of hundreds of steroid medicines, and thereby has important medicinal value. Currently, industrial production of diosgenin relies primarily on chemical extraction from plant materials. Clearly, this strategy shows drawbacks of excessive reliance on plant materials and farmland as well as environment pollution. Due to development of metabolic engineering and synthetic biology, bio-production of diosgenin has garnered plenty of attention. Although the biosynthetic pathways of diosgenin have not been completely identified, in this review, we outline the identified biosynthetic pathways and key enzymes. In particular, we suggest heterologous biosynthesis of diosgenin in *Saccharomyces cerevisiae*. Overall, this review aims to provide valuable insights for future complete biosynthesis of diosgenin.

Keywords: diosgenin, biosynthetic pathway, key enzymes, heterologous expression

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薯蓣皂苷 (Dioscin) 属于甾体皂苷，主要存在于盾叶薯蓣 *Dioscorea zingiberensis*、葫芦巴 *Trigonella foenum-graecum* 等植物中[1-2]。薯蓣皂苷由薯蓣皂素 (Diosgenin)、两个鼠李糖基和一个葡萄糖基构成 (图 1)[3]。薯蓣皂素与甾体激素类药物具有类似结构，因而具有药用价值[4]。通过对薯蓣皂素进行结构修饰，目前已得到多种甾体激素类药物[5]，这些药物具有抗肿瘤[6]、抗炎[7]、增强免疫[8]、保护肝脏[9]等功效。薯蓣皂素在植物体内含量甚微，无法满足药用需求。微生物异源合成可解决该问题。目前已异源合成多种植物次生代谢产物，例如香叶醇 (Geraniol)[10]、青蒿素前体紫穗槐二烯 (Amorphadiene)[11]、青蒿酸 (Artemisinic acid)[12]、紫杉烯 (Taxadiene)[13]、β-胡萝卜素 (β-carotene)[14]等。据报道，薯蓣皂素的生物合成分为 3 个阶段：(1) 乙酰辅酶 A 经甲羟戊酸途径 (MVA pathway) 或甲基-赤藓醇-4-磷酸途径 (MEP pathway) 分别生成异戊烯焦磷酸 (Isopentenyl diphosphate，IPP) 和二甲基烯丙基焦磷酸 (Dimethylallyl diphosphate，DMAPP)；(2) IPP 和 DMAPP 经多步酶促反应，碳链延长与环化，形成胆固醇；(3) 胆固醇经多步氧化和成环反应，生成薯蓣皂素[15]。环阿屯醇合酶 (Cycloartenol synthase，CAS)、细胞色素 P450 酶 (Cytochrome P450 enzyme，CYP450s) 和尿苷二磷酸糖基转移酶 (UDP-glucosyltransferase，UGTs) 在薯蓣皂素的合成中起关键作用[16-17]。

1 薯蓣皂素的生物合成途径

薯蓣皂素属于甾醇类物质，其合成前体为 C5 异戊二烯单位 IPP 和 DMAPP。IPP 和 DMAPP 在高等植物体内分别由 MVA 途径和 MEP 途径生成[18]。MVA 途径存在于细胞质中，在薯蓣皂素骨架形成中起主要作用[19]。MVA 途径起始于乙酰辅酶 A (Acetyl-coenzyme A，Ac-CoA)，Ac-CoA 经 6 步酶促反应，生成薯蓣皂素的基本组成单元 IPP[20]。质体中的 MEP 途径始于 3-磷酸甘油醛 (Glyceraldehyde 3-phosphate，G3P) 和丙酮酸，经 7 步酶促反应生成 IPP 和 DMAPP (图 1)。IPP 和 DMAPP 可相互转化[21]，二者在法尼基焦磷酸合酶 (Farnesyl diphosphate synthase，FPS) 催化下首尾相连，生成法尼基焦磷酸 (Farnesyl pyrophosphate，FPP)。FPP 经鲨烯合酶 (Squalene synthase，SS) 和鲨烯环氧酶 (Squalene epoxidase，SE) 连续催化，碳链延长并被氧化、环化，形成 2,3-氧化鲨烯 (2,3-oxidosqualene)[22]。2,3-氧化鲨烯是合成甾醇和三萜的分支点。2,3-氧化鲨烯经不同的氧化鲨烯环化酶 (Oxidosqualene cyclase，OSCs) 催化，形成不同结构的次生代谢产物前体 (图 2)。环阿屯醇和羊毛甾醇是甾醇类物质的合成前体，分别由 CAS 和羊毛甾醇合酶 (Lanosterol synthase，LAS) 催化形成。Mohammadi 等分析了葫芦巴 *T. foenum-graecum* 中薯蓣皂素生物合成的转录本和代谢物数据，未发现 LAS[23]，表明环阿屯醇为生物合成薯蓣皂素的中间体，又经同位素标记技术探明胆固醇为薯蓣皂素的合成前体[24]，环阿屯醇合成胆固醇的 9 个酶促步骤也已在植物分子水平上表征[25]。迄今为止，胆固醇合成薯蓣皂素的相关基因尚未完全阐明。Zhou 等用茉莉酸甲酯 (Methyl jasmonate，MeJA) 诱导葫芦巴多产薯蓣皂素，分析诱导植株与未处理植株的差异基因，发现 *CYP450* 和 *UGT* 基因参与胆固醇后期修饰步骤[26]。Christ 鉴定出葫芦巴中相关 *CYP450* 基因 (*TfCYP90B50* 和 *TfCYP82J17*)，并在酿酒酵母 *Saccharomyces cerevisiae* 中验证其功能。*Tf*CYP90B50 和 *Tf*CYP82J17 可在胆固醇 C-16 和 C-22 位加氧修饰，但未得到 C-26 位氧化产物的结构信息[16]。胆固醇 C-26 位羟基需在 UGTs 催化下添加葡萄糖基，然后裂解葡萄糖基，形成薯蓣皂素 F 环 (图 1)[27]，但参与这步反应的相关基因还需挖掘。

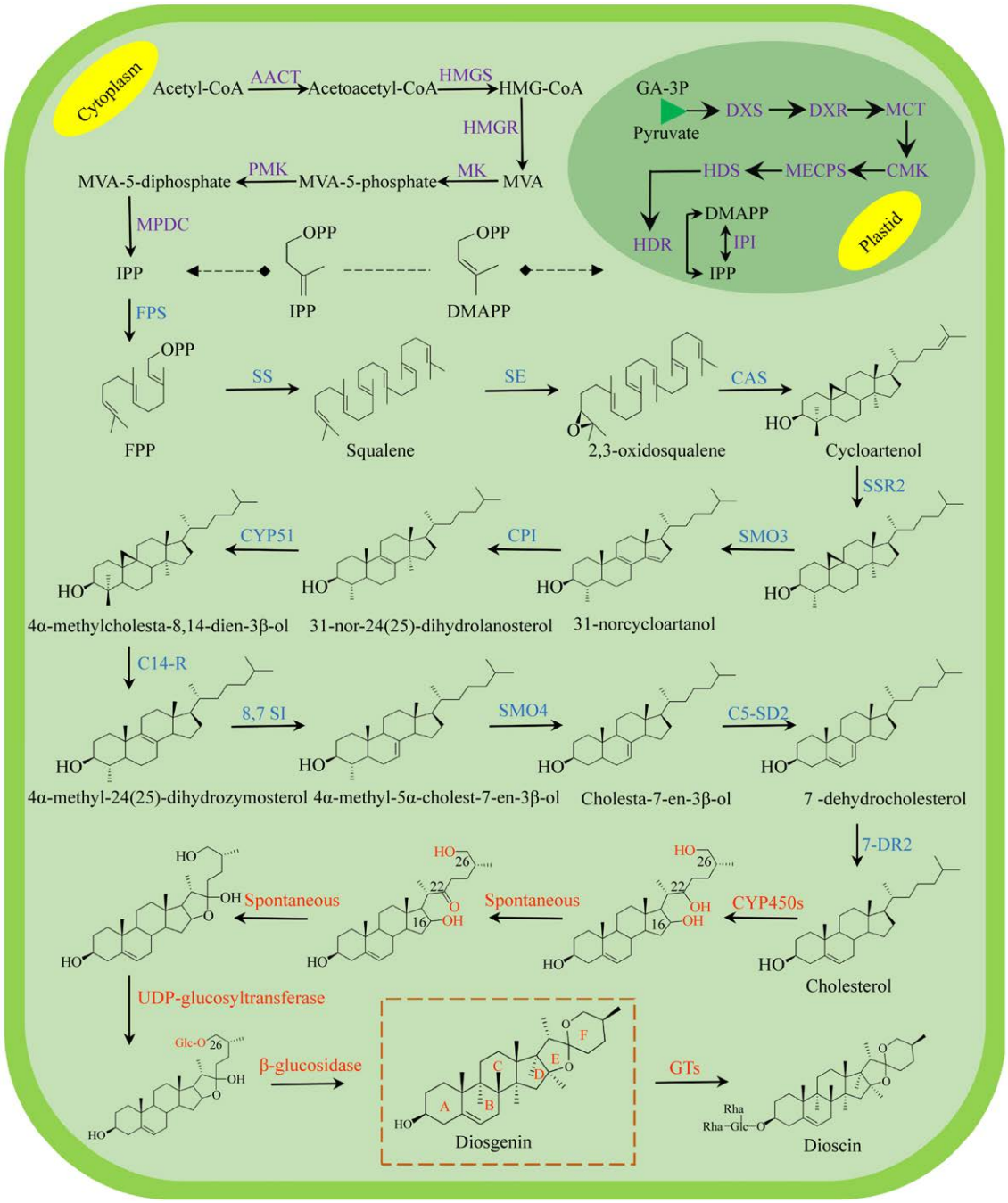

图 1 植物体内薯蓣皂素的合成途径

Fig. 1 Biosynthetic pathway of diosgenin in plants. Abbreviations: AACT: acetyl CoA acetyltransferase; HMGS: hydroxymethyl glutaryl CoA synthase; HMGR: 3-hydroxy-3-methylglutaryl-CoA reductase; MK: mevalonate kinase; PMK: phosphomevalonate kinase; MPDC: mevalonate diphosphophosphate decarboxylase; DXS: 1-deoxy-D-xylulose-5-phosphate synthase; DXR: 1-deoxy-D-xylulose-5-phosphate reductoisomerase; MCT: 2-C-methyl-D-erythritol 4-phosphatecytidylyl transferase; CMK: 4-diphospho-cytidyl-2-C-methyl-D-erythritol kinase; MECPS: 2-C-methyl-D-erythritol-2,4-cyclodiphosphate synthase; HDS: 4-hydroxy-3-methylbut-2-(*E*)-enyldiphosphate synthase; HDR: 4-hydroxy-3-methylbut-2-enyldiphosphate reductase; IPI: IPP diphosphate isomerase; SSR: sterol side chain reductase; SMO: C-4 sterol methyloxidase; CPI: cycloeucalenol cycloisomerase; CYP51: sterol C-14 demethylase; C14-R: sterol C-14 reductase; 8,7-SI: sterol 8,7-isomerase; C5-SD: sterol C-5 (6) desaturase; 7-DR: 7-dihydrocholesterol reductase.

2 关键酶分析

2.1 环阿屯醇合酶

2,3-氧化鲨烯在 OSCs 家族的催化下，可在不同位置脱质子，形成碳正离子。碳正离子经重排，2,3-氧化鲨烯可形成多种不同结构的甾醇类和三萜类化合物前体 (图 2)。CAS 属于 OSCs 家族，可催化 2,3-氧化鲨烯在 C-20 位脱质子，形成原甾醇碳正离子 (Protosteryl cation)，之后 C^+-20 重新排布到 C-9 位，并在此位发生成环反应，2,3-氧化鲨烯形成环阿屯醇[22,28]。迄今为止，CAS 催化机理未明。在拟南芥 *Arabidopsis thaliana* CAS 研究中，通过定向进化与随机诱变，发现 Tyr410、His477 和 Ile481 是其催化功能的关键残基[29-31]。植物 CAS 序列高度保守，具有保守结构域 Asp-Cys-Thr-Ala-Glu (DCTAE)[32]。DCTAE 序列可影响 CAS 与底物的结合与环化[33-34]。Mashayekh 等从葫芦巴中克隆出了编码 756 个氨基酸的 *CAS* 基因 (GenBank 登录号：APA19295.1)。*Tf*CAS 与已知 CAS 序列的相似性超过 80%，且具有保守结构域 DCTAE。目前植物 OSCs 家族的晶体结构尚未报道，仅有人类 OSCs 三维结构被解析 (PDB ID: 1W6J (图 3B)；1W6K (图 3C))。人类 OSCs 由两个环形相连的桶状 α 结构域和 3 个小的 β 结构域组成[34]。生物合成薯蓣皂素需异源表达 *CAS* 基因，同时降低 *LAS* 的表达可避免竞争性抑制，减少羊毛甾醇合成通量，使更多的 2,3-氧化鲨烯流向 CAS 通路[35]。此外，过表达宿主 MVA 途径中的 HMGR、FPS、SS 和 SE，也可增加 2,3-氧化鲨烯的产量[36-37]。

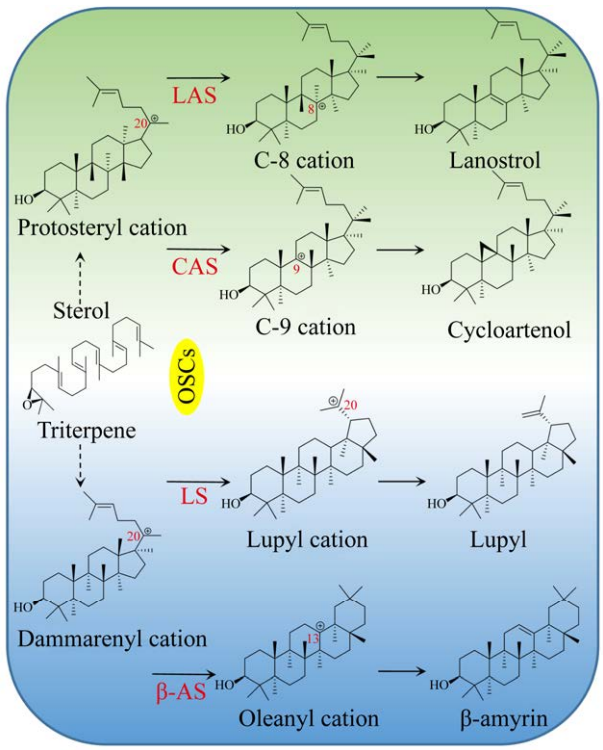

图 2 2,3-氧化鲨烯代谢支路

Fig. 2 2,3-oxidosqualene metabolic pathway. LS: lupyl synthase; β-AS: β-amyrin synthase.

2.2 细胞色素 P450 酶

CYP450 是一个超基因家族，编码的单加氧酶可对底物特异性位点加氧修饰，参与多种次生代谢产物的后修饰。在薯蓣皂素生物合成中，CYP450s 对胆固醇 C-22、C-16 和 C-26 位置氧化修饰[26]。目前已有多种植物 CYP450s 被分离鉴定，但参与生物合成薯蓣皂素 CYP450s 的报道很少。CYP450s 是结合于内质网的氧化还原酶，需 NADPH 依赖型还原酶 (NADPH-dependent reductase) 为其传递电子。异源表达 CYP450s 需

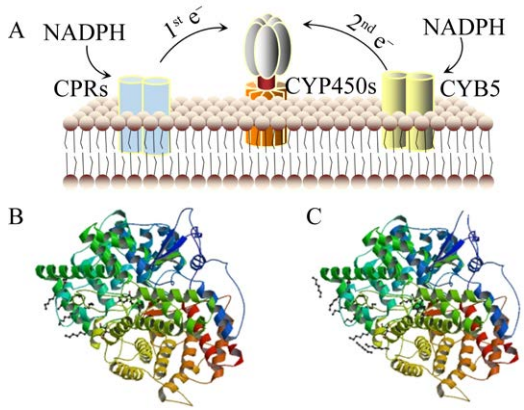

图 3 CYP450s 电子传递体系 (A) 和人类 OSCs (B–C) 的三维结构

Fig. 3 Structures of the electron transport system of CYP450s (A) and human OSCs (B–C).

解决两大问题：其一，保证 CYP450s 正确折叠；其二，保证 CYP450s 有效的电子传递效率。大肠杆菌 *Escherichia coli* 缺乏内质网，CYP450s 无法正确锚定内膜系统，导致包涵体的形成。N-末端工程可解决此问题。Biggs 等将 CYP450s N-末端替换为来自牛 (Bovine) 的亲水性八残基序列肽 (8RP)，实现了 CYP450s 在大肠杆菌 *E. coli* 中的正常表达[38]。8RP 已成为 N-末端工程的普选序列。以酿酒酵母为宿主，也可对 CYP450s N-末端修饰，以保证 CYP450s 的高效折叠[39]。CYP450s 在异源宿主内维持高催化效率，需为其设计有效的电子供体。还原力不足会降低 CYP450s 系统的催化效率；而还原当量过剩导致有毒中间体的产生。CYP450s 与 CYP450 还原酶 (Cytochrome P450 reductase，CPRs) 需优化配对。据报道，CYP450s 表达量通常高于 CPRs[40]，且 CYP450s 与细胞色素 b_5 (Cytochrome b_5，CYB5) 共表达可提高 CYP450s 系统反应效率[41] (图 3A)。基于上述研究，Paddon 等在酿酒酵母中异源表达黄花蒿 *Artemisia annua* 的 *CYP71AV1*、*CPR1* 和 *CYB5* 基因，以启动子强弱来控制 CYP71AV1、CPR1 和 CYB5 的表达量，研究其最佳配比，保证 CYP450s 系统的催化效率[12]。不同菌株及载体也会影响 CYP450s 系统的催化效率，Hausjell 等进行了探索[42]，但难点仍是 CYP450s 系统的电子通量。Nielsen 等通过异源酶亚细胞区域化策略 (Subcellular compartmentalization)，将高粱 *Sorghum bicolor* 的 CYP79A1、CYP71E1 和 UGT85B1 表达于烟草 *Nicotiana benthamiana* 的叶绿体，利用烟草光合作用产生的还原力，启动 CYP450s 系统[43]。植物光合作用以铁氧还原蛋白 (Ferredoxins) 为电子载体。铁氧还原蛋白处于光合还原力中心，与异源 CYP450s 系统存在还原当量竞争。Mellor 等比较了 3 种植物铁氧还原蛋白和一种类黄素工程蛋白电子载体 (Flavodoxin-like engineered protein) 对 CYP450s 系统的催化效率，发现以类黄素工程蛋白为电子载体，可减小还原力内源竞争，为异源 CYP450s 系统提供最佳的氧化还原电位[44]。

2.3 尿苷二磷酸糖基转移酶

糖基转移酶 (Glucosyltransferase，GTs) 是一个蛋白质超家族，催化糖基转移到甾醇、三萜等特定受体分子。根据氨基酸序列相似性、催化机制和保守序列，GTs 可分为 110 个家族 (http://www.cazy.org/GlycosylTransferases.html)。UGTs 属于 GTs 家族 1，它以尿苷二磷酸 (Uridine diphosphate UDP) 活化的糖分子为供体，将葡萄糖、鼠李糖、半乳糖、阿拉伯糖、木糖和葡萄糖醛酸等添加至受体分子的特定位置[22]。植物中存在大量 *UGT* 基因，例如，已在拟南芥 *A. thaliana* 基因组中鉴定出 107 个 *UGT* 基因[45]，其中 *UGT73C6*、*UGT78D1*、*UGT74F2* 和 *UGT75C1* 的功能已明确[46-48]，但参与薯蓣皂素合成的 *UGT* 基因尚未报道[26]。已报道的 UGTs 序列同源性相对较低，蛋白结构高度相似。目前已有 10 个植物 UGTs 晶体结构被解析 (表 1)，这些植物 UGTs 晶体结构显示其均含有 GT-B 型折叠 (GTs 家族两种普遍折叠之一)。

这种 GT-B 型折叠包含与 Rossmann 折叠结构类似的两个 C-末端和两个 N-末端结构域。每个结构域包含一个携带双侧 α 螺旋的 β 折叠。UGTs 的 N-和 C-末端结构域都形成可容纳底物结合的凹陷。核苷酸糖供体与 UGTs C-末端结构域结合，受体分子与 N-末端结构域结合。不同 UGTs C-末端结构域高度相似，包含保守序列 PSPG (Plant secondary product glycosyltransferase)。PSPG 序列含 44 个氨基酸残基，核苷酸糖供体主要与此基序中的残基作用[49]。UDP-葡萄糖 (UDP-glucose，UDP-glu) 主要与位于 PSPG 最后一位的谷氨酰胺互作[50]。PSPG 关键残基对特异性供体的识别至关重要。Han 等将 UGT78D3 的 PSPG 基序进行单点突变 (H380Q)，UGT78D3 由识别 UDP-阿拉伯糖转为识别 UDP-glu 和 UDP-木糖[51]。不同 UGTs

表 1 已解析晶体结构的尿苷二磷酸糖基转移酶
Table 1 The UGTs of resolved crystal structures

Protein	Organism	GenBank	Uniprot	PDB
UGT72B1	*Arabidopsis thaliana*	CAB8091.6	Q04622	2VCE
UGT74F2	*Arabidopsis thaliana*	AAB64024.1	O22822	5U6M
UGT89C1	*Arabidopsis thaliana*	AAF80123.1	Q8LGD9	6IJ7
UGT78K6	*Clitoria ternatea*	BAF49297.1	A4F1R4	3WC4
UGT71G1	*Medicago truncatula*	AAW56092.1	Q5IFH7	2ACV
UGT85H2	*Medicago truncatula*	ABE87250.1	A6XNC5	2PQ6
UGT78G1	*Medicago truncatula*	ABI94025.1	A6XNC6	3HBF
UGT74AC1	*Siraitia grosvenorii*	AEM42999.1	–	6L8W
UGT76G1	*Stevia rebaudiana*	AAR06912.1	Q6VAB4	6INF
*Vv*GT1	*Vitis vinifera*	AAB81683.1	O22304	2C1X

N-末端结构域差异较大，可结合类型多样的糖基受体。结构修饰 N-末端可影响 UGTs 催化效率和糖基化位点。以山奈酚 (Kaempferol) 为受体，单点突变 (I305T) 使 UGT85H2 催化效率提高 37 倍[52]。通过域交换策略 (Domain-swapping) 可设计酶嵌合体，改变酶特性。Cartwright 等设计了 UGT74F1 与 UGT74F2 嵌合体，改变了针对槲皮素 (Quercetin) 的糖基化位点[53]。基于结构酶工程策略，设计突变体与嵌合体，可改变 UGTs 的催化效率、底物特异性和区域选择性。

3 异源合成薯蓣皂素的途径设计与改造策略

异源合成薯蓣皂素需首先选择合适宿主。拟南芥和烟草遗传转化体系成熟，是理想表达宿主。Schnee 等在拟南芥中异源表达玉米萜烯合酶 (TPS10)，成功合成了 (E)-β-金合欢烯 ((E)-beta-farnesene)[54]。在烟草中也成功构建萜类[55]、黄酮类[56]等植物次生代谢产物合成途径。模式植物虽可为植物次生代谢产物的异源合成提供相似细胞环境，但生长缓慢、代谢复杂。藻类也是理想的表达宿主，可通过光合作用固定 CO_2 并为 CYP450s 代谢通路提供还原力，且同时存在 MVA 和 MEP 途径 (其他异养微生物体内只存在其中一条)，但光合固碳的效率低，严重制约次级产物的合成能力[57-59]。因此，可选用生长快且易培养的大肠杆菌和酿酒酵母。目前大肠杆菌已用于生产青蒿素前体紫穗槐二烯[11]、紫杉烯[13]、β-胡萝卜素[14]等高值药物。与大肠杆菌相比，酿酒酵母可为 CYP450s 提供内质网等内膜系统，以便参与次生代谢产物的后修饰步骤。同时，作为模式真核生物，酿酒酵母异源表达植物源基因有更好的适配性。Paddon 等利用酿酒酵母生产青蒿酸，产量达 25 g/L[12]。Galanie 等在酿酒酵母中成功合成二甲基吗啡 (Thebaine) 和氢可酮 (Hydrocodone)[60]。科学家虽已深入探讨薯蓣皂素的上游合成途径，但胆固醇的下游修饰步骤仍缺乏详细研究。根据植物基因组和转录组数据库，利用诸如 plantiSMASH[61]和 1KP Project[62]计算方法分析不同植株表达差异、挖掘关键基因使得在酿酒酵母中构建薯蓣皂素全生物合成途径成为可能[16,63] (图 4)。目前已构建多个平台菌。Meadows 等构建了高产 FPP 酿酒酵母 (130 g/L)[64]，Souza 等构建了产胆固醇的酿酒酵母菌株 RH6829[63]，二者均可作为薯蓣皂素的生产宿主。

薯蓣皂素的异源合成途经长，需保证多步骤间整体催化效率，而实现高催化效率需充足的底物供应。Ac-CoA 是合成薯蓣皂素的前体物，在酵母菌中过表达合成 Ac-CoA 的关键基因可增加其产量。Zhao 等过表达 NADP 依赖型醛脱氢酶基因

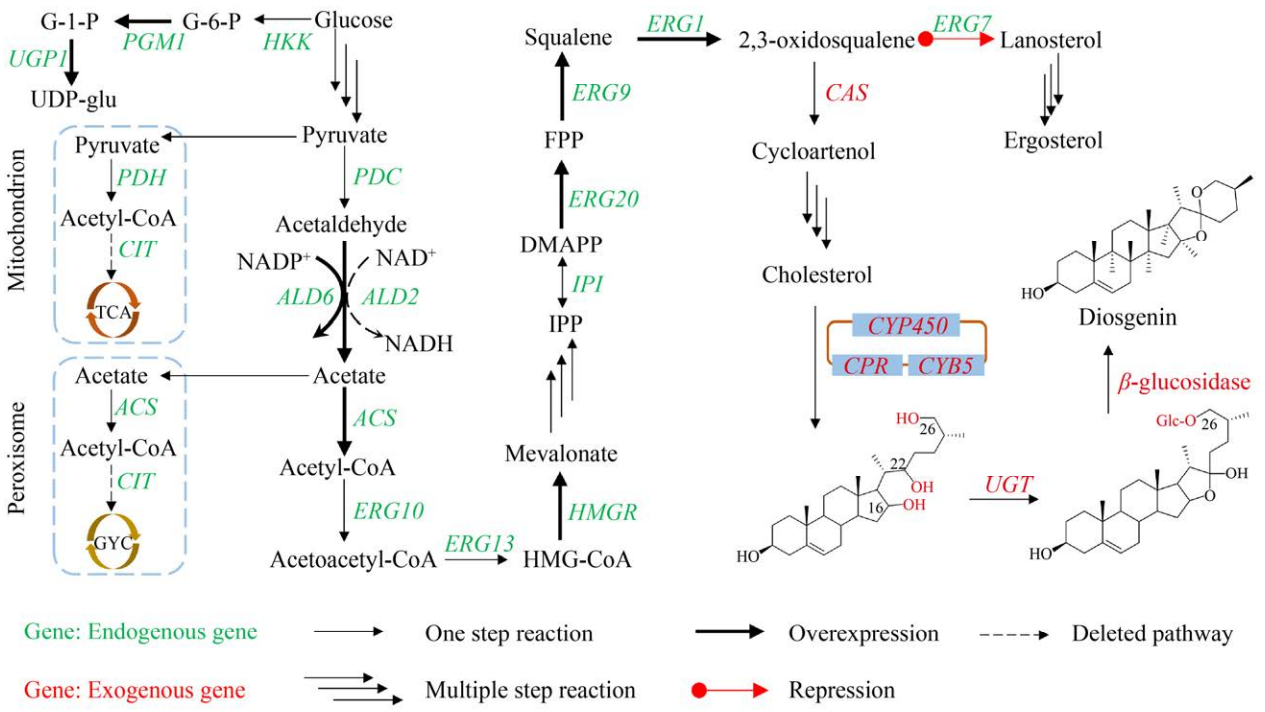

图 4　酿酒酵母中构建薯蓣皂素合成途径

Fig. 4　Protocol for engineering biosynthetic pathway of diosgenin in *S. cerevisiae*. G6P: glucose 6-phosphate; G1P: glucose 1-phosphate; PDH: pyruvate dehydrogenase; PDC: pyruvate decaboxylase.

ALD6，并引入肠道沙门氏菌 *Salmonella enterica* 的 Ac-CoA 合成酶基因 $ACS_{seL641P}$ (Acetyl-CoA synthase)，提高了 Ac-CoA 产量[65]。酵母菌细胞质产生的 Ac-CoA 可进入三羧酸循环 (Tricarboxylic acid cycle，TCA) 和过氧化物酶体乙醛酸循环 (Glyoxylate cycle，GYC)。敲除柠檬酸合酶基因 *CIT* (Citrate synthase) 可抑制 Ac-CoA 进入 TCA 和 GYC 循环，促使 Ac-CoA 流向 MVA 途径[66]。UDP-glu 是合成薯蓣皂素的关键底物。葡萄糖由己糖激酶 (Hexokinase，HXK1)、葡萄糖磷酸变位酶 (Phosphoglucomutase，PGM1) 和葡萄糖-1-磷酸尿苷转移酶 (Glucose-1 phosphate uridylyltransferase，UGP1) 催化，产生 UDP-glu。过表达 PGM1 和 UGP1 可提高 UDP-glu 产量[67]。维持高催化效率还需要充足的辅因子。NADPH 是生物合成薯蓣皂素的主要辅因子。乙醛脱氢酶 (Aldehyde dehydrogenases) 催化乙醛转化为乙酸，由 *ALD6* 或 *ALD2* 编码。ALD6 以 $NADP^+$为辅因子，ALD2 以 NAD^+为辅因子[68]。Kim 等在酿酒酵母中过表达 *ALD6* 并敲除 *ALD2*，提高了细胞质中 NADPH 的浓度，目标产物原人参二醇 (Protopanaxadiol) 产量随之提高[69]。维持高催化效率还需抑制有毒中间体的产生。设计敏感调控元件，响应细胞内外有毒中间体的浓度变化，可动态调控代谢流。Dahl 等分析鉴定出对有毒中间体 FPP 敏感的启动子，并将其整合到紫穗槐二烯异源表达途径中。筛选的启动子可对 FPP 上下游途径实施反馈调节，使得 FPP 积累减少，紫穗槐二烯产量提高 2 倍[70]。

4　总结与展望

薯蓣皂素作为数百种类固醇药物的前体，具有重要药用价值。由于化学提取法作为现今其主要生产手段，存在产率较低且污染环境等问题，所以生物合成法日益受到关注。目前，薯蓣皂素

的基因工程全合成鲜有报道。植物宿主生长缓慢，代谢网络复杂，改造难度大；酵母作为宿主虽有成功案例，但各步骤间适配性差，产量低，无法工业化生产[16]。因此，需利用代谢工程和合成生物学最新技术手段对薯蓣皂素的生物合成进行系统研究。迄今面临的困难主要有以下几点：(1) 关键酶的催化效率低；(2) 产物合成途径长；(3) 异源途径与底盘细胞的适配性差；(4) 目的产物或中间代谢物积累造成胁迫，限制产量的提高。

为构建稳定高效的微生物细胞工厂，需从酶、途径、代谢网络、细胞等不同维度研究其适配与平衡的关系。因此提出以下研究方向：(1) 利用结构生物学和计算生物学手段，筛选并建立关键酶库 (如 CYP450s 和 UGTs)。通过比较不同酶的催化差异，揭示关键酶催化效率和专一性的分子机制，进而建立理性设计酶分子的策略。(2) 针对多步催化的长合成途径，建立并完善基因组整合机制。利用 CRISPR 和 Red 重组等技术对宿主基因组进行编辑，构建整合位点库，为长途径的分散高效组装奠定基础[71-72]。(3) 探究异源途径在底盘细胞中的最优表达策略，利用 N-末端工程、信号肽工程实现胞内关键酶的空间定位[73]；建立以 RNA 聚合酶与核糖体为枢纽的正交表达系统，将外源途径与底盘细胞的自身代谢网络分割开来，实现细胞资源在不同途径间的合理分配[74]。(4) 针对某些中间代谢物或终产物的细胞毒性大且造成反馈抑制等问题，开发代谢物传质及转运技术，可利用蛋白支架将多步酶捆绑，强化中间物的转化效率；同时设计转运蛋白，以减少胞内代谢物积累，解除胁迫效应。随着代谢工程及合成生物学策略的运用，薯蓣皂素的大规模发酵生产必将实现。

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