·综 述·

酵母甾醇转运蛋白研究进展

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王钰, 武陶, 樊旭倩, 阮海华, 樊飞宇, 张学礼. 酵母甾醇转运蛋白研究进展[J]. 生物工程学报, 2023, 39(8): 3204-3218.
WANG Yu, WU Tao, FAN Xuqian, RUAN Haihua, FAN Feiyu, ZHANG Xueli. Sterol transport proteins in yeast: a review[J]. Chinese Journal of Biotechnology, 2023, 39(8): 3204-3218.

摘 要：甾醇是一类广泛存在于生物体内的环戊烷骈多氢菲衍生物，其不仅是细胞膜的重要组成成分，还具有重要的生理和药理活性。随着合成生物学和代谢工程技术的发展，近些年来应用酵母细胞异源合成甾醇的研究不断深入。但由于甾醇是疏水性大分子，倾向于积累在酵母的膜结构中而引发细胞毒性，一定程度上限制了甾醇产量的进一步提升。因此，揭示酵母中甾醇转运机制，特别是与甾醇转运相关的转运蛋白的工作原理，有助于设计新的策略，解除酵母细胞工厂中的甾醇积累毒性、实现甾醇增产。酵母中甾醇转运主要通过蛋白质介导的非囊泡运输机制来完成，本文归纳了酵母中已报道的5类甾醇转运相关蛋白，即OSBP/ORPs家族蛋白、LAM家族蛋白、NPC样甾醇转运蛋白、ABC转运家族蛋白和CAP超家族蛋白，汇总了这些蛋白对细胞内甾醇梯度分布和稳态维持所起的重要作用。此外，本文还综述了甾醇转运蛋白在酵母细胞工厂中的应用现状。

关键词：甾醇；甾醇转运；转运蛋白；酵母；合成生物学

资助项目：国家重点研发计划(2019YFA0905300)；天津市合成生物技术创新能力提升行动(TSBICIP-KJGG-001)；国家自然科学基金(32225031，32271482，21908168)；天津市研究生科研创新项目(2021YJSS299)
This work was supported by the National Key Research and Development Program of China (2019YFA0905300), the Tianjin Synthetic Biotechnology Innovation Capacity Improvement Project (TSBICIP-KJGG-001), the National Natural Science Foundation of China (32225031, 32271482, 21908168), and the Tianjin Research Innovation Project for Postgraduate Students (2021YJSS299).
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Received: 2023-01-17; Accepted: 2023-04-27; Published online: 2023-05-17

Sterol transport proteins in yeast: a review

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Abstract: Sterols are a class of cyclopentano-perhydrophenanthrene derivatives widely present in living organisms. Sterols are important components of cell membranes. In addition, they also have important physiological and pharmacological activities. With the development of synthetic biology and metabolic engineering technology, yeast cells are increasingly used for the heterologous synthesis of sterols in recent years. Nevertheless, since sterols are hydrophobic macromolecules, they tend to accumulate in the membrane fraction of yeast cells and consequently trigger cytotoxicity, which hampers the further improvement of sterols yield. Therefore, revealing the mechanism of sterol transport in yeast, especially understanding the working principle of sterol transporters, is vital for designing strategies to relieve the toxicity of sterol accumulation and increasing sterol yield in yeast cell factories. In yeast, sterols are mainly transported through protein-mediated non-vesicular transport mechanisms. This review summarizes five types of sterol transport-related proteins that have been reported in yeast, namely OSBP/ORPs family proteins, LAM family proteins, ABC transport family proteins, CAP superfamily proteins, and NPC-like sterol transport proteins. These transporters play important roles in intracellular sterol gradient distribution and homeostasis maintenance. In addition, we also review the current status of practical applications of sterol transport proteins in yeast cell factories.

Keywords: sterol; sterol transport; transport proteins; yeast; synthetic biology

甾醇(sterol)又称固醇，是一类含有环戊烷骈多氢菲(cyclopentano-perhydrophenanthrene)母核结构的化合物，几乎所有生物体都能自身合成。其中胆固醇(cholesterol)、菜油甾醇(campesterol)及麦角固醇(ergosterol)分别为动物、植物及真菌细胞膜的主要成分。除了膜组分甾醇，动植物中还含有其他种类的甾醇，如植物体内的豆甾醇(stigmasterol)、薯蓣皂素(diosgenin, DSG)、β-谷甾醇(β-sitosterol)等；动物细胞内含有的维生素 D、黄体酮、皮质醇和胆酸等[1]。甾醇是细胞膜、激素、信号分子以及与防御相关的生物和非生物化学物质的重要组成部分，也是包括类固醇和羟基甾醇在内的多类信号分子的前体[2]。以天然的甾醇为前体，在其母核或侧链上进行不同的基团修饰可获得抗炎、抗感染、抗过敏、抗病毒和抗休克等不同药理学活性的甾体激素类药物，如氢化可的松、地塞米松等。

甾醇在细胞内通常以游离甾醇(free sterol)与甾醇酯(sterol ester)两种形式存在，它们之间的酯化与水解可以动态平衡游离甾醇的含量[3-4]。甾醇的合成主要发生在内质网(endoplasmic reticulum, ER)，但内质网的甾醇含量极低，约90%的游离甾醇积累在细胞膜上，占细胞膜脂质的约 30 mol%[5]。酿酒酵母是兼性厌氧生物，有

氧条件下在内质网中合成麦角固醇，缺氧时面临甾醇缺陷则需从环境中吸收甾醇。摄取的游离甾醇被运输至内质网，其中一部分随后被运送至质膜，成为细胞膜的主要成分；另一部分则与长链脂肪酸发生酯化反应后贮存于脂滴中[5-7]。

甾醇作为亲脂性化合物，其转运方式可归纳为：囊泡运输和非囊泡运输[8]。研究发现，抑制囊泡运输对甾醇向细胞膜的转运影响甚微，因此普遍认为细胞器膜间甾醇交换主要通过甾醇转运蛋白(sterol transport proteins, STPs)介导的非囊泡运输[7,9]。STPs 可能是细胞质蛋白、膜结合蛋白或分泌蛋白，它们的作用可以是从一类膜结构中摄取甾醇途经细胞质将其转运至另一类膜结构上，也可以是在膜结合位点(membrane contact site, MCS)处发挥作用，或者是结合胞内游离甾醇通过自身分泌将甾醇携带至细胞外[8,10]。

1 酵母甾醇转运蛋白 STPs

酵母的非囊泡甾醇转运主要依赖于 5 类蛋白(表 1)，即氧化甾醇结合蛋白相关蛋白(oxysterol-binding protein-related proteins, ORPs)、锚定在细胞膜接触位点的脂交换家族蛋白(lipid transfer proteins anchored at the membrane contact sites, LAMs)、NPC 样甾醇转运蛋白[Niemann-Pick disease type C2 protein (NPC)-like sterol transport protein]、ABC 转运蛋白家族(ATP-binding cassette transporter, ABC)、CAP 蛋白超家族[cysteine-rich secretory proteins (CRISP), antigen 5 (Ag5), and pathogenesis-related 1 (PR-1) proteins, CAP][44]。5 类甾醇转运蛋白基本作用方式如图 1 所示，甾醇在亚细胞结构之间的非囊泡运输主要依赖 OSBP/ORPs 和 LAM 蛋白家族；NPC 样甾醇转运蛋白主要负责甾醇的再分配；ABC 转运蛋白家族负责甾醇的外排以及厌氧条件下外源甾醇的摄取；CAP 蛋白超家族负责甾醇向胞外分泌。

1.1 氧化甾醇结合蛋白相关蛋白家族 OSBP/ORPs

氧化甾醇结合蛋白相关蛋白[oxysterol-binding protein (OSBP)-related proteins, ORPs]是一类广泛分布于生物体中的保守蛋白家族，从酵母到人类都有其存在。该家族的成员自 35 年前被首次发现后，其在膜脂和甾醇转运中的功能逐步得到揭示[45-46]。所有 OSBP/ORPs 家族的成员蛋白的共同特征是 C 端含有一个 OSBP 相关配体结合结构域 ORD (OSBP-related ligand-binding domain, ORD)，该结构域由 β 折叠构成核心，形成一个外部亲水、内部疏水的桶状通道[47]。

酿酒酵母中有 7 个 OSBP/ORPs 家族成员蛋白，分别命名为 Osh1p−Osh7p。它们能在亚细胞结构之间转运甾醇，并严格调控不同膜结构上的甾醇分布。该转运过程一般发生在膜结合位点，即 2 个细胞器膜之间距离小于 30 nm 的区域[48]。以 Osh4p 为例，研究表明其 ORD 在体外能与 5 种不同的甾醇结合。当 Osh4p 与甾醇结合时，甾醇极性头部朝下将 C3-羟基埋在通道底部。此时 Osh4p 的 N 端形成一个类似盖子的结构，它会关闭以稳定当前蛋白构象，此举有利于 Osh-甾醇复合体通过水屏障以及传输传递信号[13,49]。Osh1p−Osh7p 可在内质网-高尔基体、内质网-线粒体之间传递甾醇与脂质[19,50]，也能在体外转运胆固醇[15]。Raychaudhuri 等[14]发现酿酒酵母在厌氧条件下外源摄入胆固醇和麦角固醇后，Osh1p−Osh7p 能将这些外源摄取的甾醇从细胞膜转运至内质网。其中 Osh4p 对甾醇的转运速度受到磷酸肌醇含量(phosphoinositides, PIPs)的影响：富含 PIPs 时 Osh4p 转运甾醇的速度较快；PIPs 合成途径缺陷时则甾醇转运速度显著减缓[14]。敲除 *OSH3* 或 *OSH5* 基因会导致外源胆固醇的相对酯化率减半，说明它们的功能缺失降低了甾醇

表 1 文中所提及的酵母甾醇转运蛋白的亚细胞定位、功能以及胞内转运甾醇的种类

Table 1 Subcellular localization and substrates of sterol transporters as mentioned in this review

Famliy	Name	UniProt ID	Subcellular location	Substrate	Reference
OSBP/ORPs	Osh1p	P35845	ER, GA, NoM, VM	Lipids, ergosterol, cholesterol	[11-14]
	Osh2p	Q12451	ER, PM	Lipids, ergosterol, cholesterol	[11,14-16]
	Osh3p	P38713	ER, CP	Lipids, ergosterol, cholesterol	[11,14,15]
	Osh4p	P35844	GA, CP	Lipids, ergosterol, cholesterol	[14,17-19]
	Osh5p	P35843	VM, bud neck	Lipids, ergosterol, cholesterol	[14,16,20]
	Osh6p	Q02201	ER, PM, CP	Lipids, ergosterol, cholesterol	[14-16,21]
	Osh7p	P38755	ER, PM, CP	Lipids, ergosterol, cholesterol	[14-16,22]
LAM	Lam1p	P38851	ER, MM	Lipids, ergosterol, cholesterol	[23,24]
	Lam2p	Q18823	BM	Lipids, ergosterol, cholesterol	[23,25]
	Lam3p	P38717	ER	Lipids, ergosterol, cholesterol	[23]
	Lam4p	P38800	ER	Lipids, ergosterol	[23,25]
	Lam5p	P43560	ER	Lipids, ergosterol	[23]
	Lam6p	Q08001	ER	Lipids, ergosterol, cholesterol	[23,26]
RND	Ncr1p	Q12200	VM	Ergosterol, cholesterol	[27,28]
	Ncp2p	Q12408	V	Ergosterol, cholesterol	[24,28]
ABC	Snq2p	P32568	PM	Progesterone, estradiol, phospholipid	[29-31]
	Dan1p	P47178	Secreted, CW	Ergosterol	[32,33]
	Aus1p	Q08409	PM	Ergosterol, cholesterol	[29,34]
	Pdr5p	P33302	PM	Progesterone, estradiol	[30,31,35]
	Pdr11p	P40550	PM	Ergosterol, cholesterol	[34,36]
	Pdr18p	P53756	PM	Ergosterol	[37]
	Ybt1p	P32386	VM	Bile acid	[38]
	Ycf1p	P39109	VM	Testosterone, progesterone	[39,40]
	Pdr16p	P53860	ER, MM, LD	Lanosterol, phospholipid	[41,42]
CAP	Pry1p	P47032	Secreted	Cholesteryl acetate, fatty acid	[3]
	Pry2p	P36110	Secreted	Cholesteryl acetate, fatty acids	[3]
	Pry3p	P47033	Secreted, CW	Cholesteryl acetate, fatty acids	[43]

ER: Endoplasmic reticulum; GA: Golgi apparatus; NoM: Nucleus outer membrane; VM: Vacuole membrane; PM: Plasmic membrane; CP: Cytoplasm; MM: Mitochondrion membrane; BM: Basement membrane; V: Vacuole; CW: Cell wall; LD: Lipid droplet.

从细胞膜向内质网的转运效率[14]。此外，有研究表明敲除 7 个 *OSH* 基因导致新合成的麦角甾醇从内质网到细胞膜的转移量降低到原来的 1/20[13,51]，也有报道显示只降低到原来的 1/5[52]，进一步佐证了 Osh1p−Osh7p 参与了甾醇在内质网和细胞膜之间的转运。

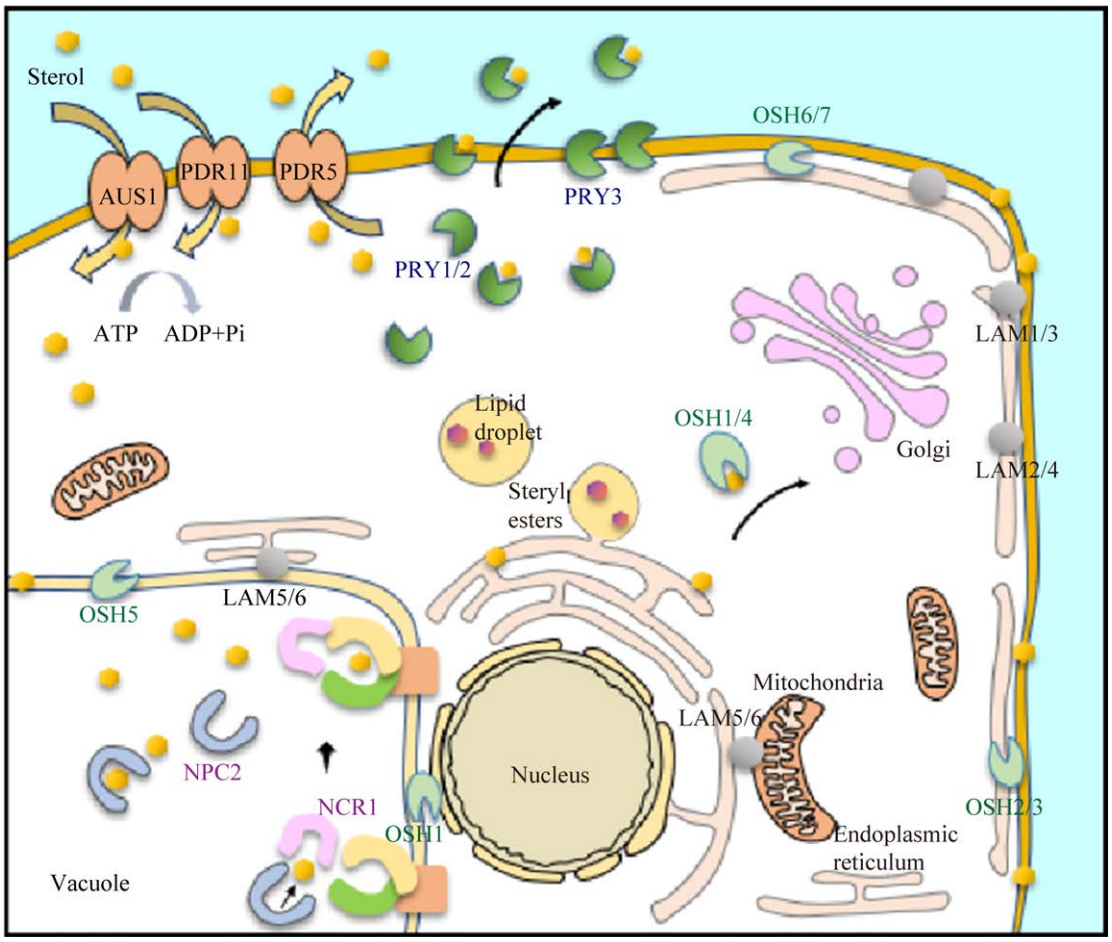

图 1　酵母细胞内甾醇转运的关键途径及蛋白　OSH1–7：氧化甾醇结合蛋白相关蛋白；LAM1–6：锚定在细胞膜接触位点的脂交换家族蛋白；NCR1：尼曼-匹克 C 型相关蛋白 1；NPC2：尼曼-匹克 C 型相关蛋白 2；AUS1：ATP 依赖性渗透酶；PDR11：ATP 依赖性渗透酶；PDR5：多效耐药转运蛋白；PRY1–3：病原相关酵母蛋白

Figure 1　The key pathways and proteins involved in sterol transport in yeast. OSH1–7: Oxysterol-binding protein homolog 1–7; LAM1–6: Membrane-anchored lipid-binding protein; NCR1: Niemann-pick type C-related protein 1; NPC2: Niemann-pick type C-related protein 2; AUS1: ATP-dependent permease; PDR11: ATP-dependent permease; PDR5: Pleiotropic ABC efflux transporter of multiple drugs; PRY1–3: Pathogenesis-related yeast protein 1–3.

1.2　锚定在胞膜接触位点的脂交换家族蛋白 LAMs

酵母中锚定在细胞膜接触位点的脂交换家族蛋白 LAMs 属于 StARkin [relatives (kin) of steroidogenic acute regulatory protein, StARkin] 超家族成员，它们参与内质网与其相邻膜结构之间甾醇的转运和调节[53]。在哺乳动物中，StARkin 超家族中的重要成员是甾体激素合成急性调节蛋白 StAR (steroidogenic acute regulatory protein, StAR)，它控制着甾体激素合成过程中第一个限速步骤[54]。在人体中，StAR 负责将游离胆固醇由线粒体外膜转运至内膜，胆固醇侧链裂解酶 (cytochrome P450 cholesterol side chain lyase, P450scc)在内膜处将胆固醇裂解为孕烯醇酮和 4-甲基戊醛，完成人体激素合成的第一步。在酿酒酵母中 LAMs 包含 6 个成员(Lam1p–Lam6p)，

它们在哺乳动物中的同源蛋白被称为GRAMD1a−c[55]。LAM 蛋白定位于内质网与其他细胞器膜形成的膜结合位点上：其中 Lam1p (Ysp1p)、Lam2p (Ysp2p/Ltc4p)、Lam3p (Sip3p)和 Lam4p (Ltc3p)定位在内质网与细胞膜形成的膜接触位点；而 Lam5p (Ltc2p)和 Lam6p (Ltc1p)定位在内质网-线粒体和内质网-囊泡形成的膜接触位点[16]。

LAM 蛋白的 C 端含有一个跨膜螺旋结构，使其能够锚定于内质网上。除此之外，它还含有 1–2 个 StART 样结构域(steroidogenic acute regulatory protein-related lipid transfer like, StART-like)，该结构域由 160−190 个氨基酸组成，是用于结合甾醇的疏水口袋[53]。Gatta 等[23]在原核细胞中表达并纯化了 Lam2p 和 Lam4p 的 4 个 StART 样结构域，并发现它们能够与胆固醇和麦角固醇等甾醇物质在体外发生结合。他们还发现，在酵母中敲除 Lam2p 的第 2 个 StART 结构域后导致菌株对两性霉素 B 更敏感，这说明该结构域对于将麦角固醇从内质网转运至细胞膜是必需的。此外，有研究表明 Lam2p 不仅能够运输麦角甾醇，还能够运输胆固醇和脱氢麦角甾醇[16]。据蛋白家族数据库显示：昆虫基因组中亦含有 *LAM* 和 *OSH* 同源基因，并且有数据表明昆虫细胞中的 LAM 可以实现从细胞膜到内质网方向上对外源摄取的甾醇进行逆向运输[16]。Gatta 等[23]的研究表明外源性胆固醇和脱氢麦角固醇的酯化效率并不受 *LAM4*/*5*/*6* 基因缺失的影响，但是单独敲除 *LAM1*、*LAM2* 或 *LAM3* 后其酯化效率会降低 40%−50%，这些结果表明蛋白 Lam1/2/3p 主要负责甾醇从细胞膜到内质网的反向运输。此外，Sokolov 等[56]的研究结果展示了 *LAM1/2/3/4* 四基因同时缺失会引起细胞膜上的麦角固醇含量增加，再次印证了 LAM 蛋白可参与甾醇的逆向转运。

1.3 NPC 样甾醇转运蛋白

哺乳动物细胞中存在着由溶酶体固醇穿梭蛋白 Npc2p 和膜蛋白 Npc1p 组成的 Niemann-PickC 型(NPC)系统，这 2 个蛋白属于耐药结节分化超家族(resistance nodulation and cell division, RND)，负责甾醇的再分配，在维持溶酶体甾醇稳态方面起着核心作用[57]。Npc1p 和 Npc2p 存在于晚期溶酶体，负责将胆固醇转运至溶酶体膜上，以调节胆固醇到高尔基体、质膜、内质网等不同细胞器间的运输[58-59]。Npc1p 和 Npc2p 蛋白突变会导致溶酶体中胆固醇的异常积累，如果发生在人体内则会引发尼曼匹克病(Niemann-Pick disease, NPD)[60]。在酿酒酵母中，Npc1p 的同源蛋白 Ncr1p 和 Npc2p 定位于液泡上，在细胞液泡膜筏状结构域的形成和扩展方面发挥着关键的作用[61]。

Npc1p (Ncr1p)由 13 个跨膜螺旋和 3 个位于腔内侧的可溶结构域[N-terminal domain (NTD)、domain C 和 domain I]组成，其中第 3 到第 7 个跨膜螺旋构成了一个保守的甾醇感知结构域(sterol-sensing domain, SSD)。Ncr1p 则是一个存在液泡腔内的可溶性小蛋白[57]。在液泡中，胆固醇非极性的碳氢尾部插入 Npc2p 的结合口袋后使其被包裹起来，只将羟基暴露在液泡腔内，随后 Npc2p 与 Npc1p 的 domain C 区域相互作用，将胆固醇从 domain C 和 domain I 之间的中心通道释放至 Npc1p 的 NTD 区域，进而向中心通道末端跨膜区的结合胆固醇感知结构域 SSD 转移，最终将胆固醇运送至液泡膜上[57,62]。Winkler 等利用荧光固醇脱氢麦角固醇(dehydroergosterol, DHE)活细胞成像来研究 Ncr1p 和 Npc2p 介导的固醇转运机制，发现外源摄入的 DHE 在细胞生理恢复正常后会被贮存于脂滴；而当细胞继续饥饿时，脂滴会被液泡摄入。野生型细胞中被液泡摄入的 DHE 积累于液泡膜上；而在 Ncr1p 或

Npc2p 缺失突变体中 DHE 多积累在腔内致使液泡呈现出不规则形状[28]，该结果说明了 Ncr1p 和 Npc2p 主要负责将甾醇从腔内运输到液泡膜上。Moesgaard 等[63]对 Npc2p 进行分子动力学模拟，表明其结合口袋可以适应配体的形状，并以疏水相互作用与麦角甾醇等甾醇结合。*NCR1* 被敲除后，甾醇向液泡膜的转运会受损，但并不会影响甾醇的合成、酯化以及摄取[28,64-65]。

1.4 ABC 转运蛋白家族

ABC 转运蛋白广泛存在于从原核生物到人类的所有物种中，是生命体中最丰富的转运蛋白家族之一。ABC 转运蛋白的作用原理是利用 ATP 水解产生的能量实现脂质、氨基酸、抗生素和甾醇等多种物质的跨膜运输[66]。ABC 转运蛋白分为可溶性和膜结合 2 种类型，前者只包含一个核苷酸结合域 (nucleotide-binding domains, NBDs)，主要参与 DNA 修复和基因调控；后者包含 2 个 NBDs 和 2 个跨膜结构域 (transmembrane domains, TMDs)，可通过打开和关闭跨膜结构域实现烷烃、氨基酸、抗生素和甾醇等底物的跨膜运输[29,67]。在酿酒酵母中，有 30 个 ABC 转运蛋白家族成员，其中 22 个是膜结合型[29]。

酵母中参与甾醇外排转运的 ABC 转运蛋白主要为 Snq2p 和多向耐药性蛋白 (pleiotropic drug resistance, PDR)亚家族成员 Pdr5p。Pdr5p 被报道可以外排多种毒性异质物从而赋予酵母细胞多药抗性，Snq2p 也已被证实参与了广泛的异质物的外排过程。有研究表明用人源甾体激素黄体酮处理酿酒酵母细胞后，*PDR5* 和 *SNQ2* 基因的表达丰度分别显著上调 11.3 倍和 3.1 倍，表明这 2 种转运蛋白功能可能与黄体酮的外排相关[30]。此外，Godinho 等[37]对多向耐药性蛋白亚家族另一成员 Pdr18p 研究发现，*PDR18* 敲除菌株的细胞膜通透性比野生型高，其麦角甾醇含量显著约下降至原来的 1/10，推测 Pdr18p 可能参与了麦角甾醇从内质网到细胞膜的转运，从而影响了细胞膜的通透性及稳定性。厌氧条件下，酵母因自身甾醇合成受到抑制需通过摄取外源甾醇维持细胞活力，此时以 Aus1p、Pdr11p 和 Dan1p 为代表的 ABC 转运蛋白担负起甾醇内向运输的功能。Aus1p 和 Pdr11p 参与厌氧条件下甾醇的摄取，缺乏这 2 种转运蛋白的酵母细胞不仅外源性甾醇的摄取过程会被阻断，导致不能在厌氧条件下生长[34]。此外，缺失 Aus1p 和 Pdr11p 蛋白会导致外源的脱氢麦角固醇积累在酵母细胞壁上，因此 Wüstner 等[68]认为 Aus1p 和 Pdr11p 蛋白是介导甾醇从细胞壁转移至细胞膜的必需元件。Papay 等[69]对 Aus1p 和 Pdr11p 的核苷酸结合域突变发现所得的突变体能选择性地影响酵母对外源甾醇的利用，再次证明了 Aus1p 是厌氧条件下甾醇的主要内运蛋白。还有研究表明胞壁甘露糖蛋白 Dan1p 在 Aus1p 介导的甾醇内向转运中起着协同作用[33]。除了上述介绍的 ABC 转运蛋白，位于液泡膜上的 ABC 转运蛋白 Ybt1p 和 Ycf1p 因具有胆酸转运功能也被列为重要的甾醇转运蛋白，Cvelbar 等[40,70]的研究表明这 2 个转运蛋白在类固醇解毒中发挥了重要的作用。Pdr16p (Sfh3p)也表现出对羊毛甾醇 (lanosterol)的高亲和力，并参与了羊毛甾醇的转移，*PDR16* 基因缺失会导致羊毛甾醇的显著增加[42]。

1.5 CAP 蛋白超家族

CAP 超家族也被称为 SCP 蛋白超家族 (sperm coating glycoprotein, SCP)，是以富含半胱氨酸的分泌蛋白 [cysteine-rich secretory proteins (CRISP)]、抗原 5 [antigen 5 (Ag5)]和致病相关蛋白 1 [pathogenesis-related 1 (PR-1) proteins]这 3 个创始成员命名的，包括了来自 1 500 多个物种的 4 500 多个已知成员[71]。CAP

蛋白保守存在于从酵母到人类的真核生物中，与多种生理过程息息相关[43]。几乎所有 CAP 蛋白都是分泌型糖蛋白，可在细胞外液中表现出较高的蛋白稳定性[72]。酿酒酵母中所含的 3 个 CAP 蛋白被命名为 PRY 蛋白，其中 Pry1p 和 Pry2p 为分泌型糖蛋白负责甾醇向胞外分泌，而 Pry3p 是含有糖基磷脂酰肌醇锚附着信号的细胞壁蛋白[43,73]。

CAP 超家族的所有成员均含有一个大约由 150 个氨基酸组成的 CAP 保守结构域，它采用独特的 α-β-α 三明治折叠立体结构且附近存有很多 loop 环，推测其可能与蛋白质的相互作用密切相关[74]。2012 年，瑞士弗里堡大学 Vineet Choudhary[3]发现 Pry1p 和 Pry2p 在体外结合游离胆固醇和胆固醇乙酸酯(cholesteryl acetate)，在酿酒酵母体内能结合胆固醇乙酸酯并将其分泌至胞外，Pry1p 和 Pry2p 在该分泌功能上存在冗余，而当 *PRY1* 和 *PRY2* 双敲除时胆固醇乙酸酯的分泌会完全被阻断；Pry1p 和 Pry2p 的甾醇结合和分泌功能取决于其 CAP 结构域，人来源的 CAP 蛋白 Crisp2p 在酵母体内表达时能解除 *PRY1* 与 *PRY2* 双敲突变体的甾醇分泌阻滞。酿酒酵母 Pry1p 的 CAP 结构域晶体结构已获解析，C279 保守残基决定了 CAP 结构域的稳定性，C279S 突变会导致 Pry1p 体外结合胆固醇和胆固醇醋酸酯的能力下降 3 倍[3]。前列腺分泌蛋白 Psp94p (prostate secretory protein of 94 amino acids, Psp94p)与 CAP 蛋白的相互作用会抑制 CAP 蛋白在体外结合甾醇。在酿酒酵母体中，Psp94p 与 Pry1p、Crisp2p 共表达虽不能阻断其分泌但能明显地阻碍其甾醇结合功能，导致甾醇分泌量减少了 78%以上[75]，这一结果表明 Psp94p 能特异性地影响 CAP 结构域的甾醇结合位点。Pry1p 对底物的选择并不特异，除了能结合胆固醇及胆固醇醋酸酯外，还可以结合麦角固醇和谷甾醇等甾体化合物[3]。

2 甾醇转运蛋白在酵母细胞工厂中的应用现状

甾体药物作为仅次于抗生素以外的第二大药物，目前全球已经批准的甾体药物超过 400 种，年销售额超过 100 亿美元[76]。一些甾醇可作为甾体药物的重要前体，但由于结构复杂而来源受限。传统提取工艺需要从薯蓣属黄姜等植物中提取甾体母核，再通过化学反应及微生物转化过程获得甾醇药物中间体，该过程合成路线资源依赖度高且会产生大量酸性废水，严重污染我国南方的水体环境[77]。而合成生物学可以利用简单碳源合成复杂化合物，符合绿色化学的观念。酿酒酵母因具有遗传背景清晰、基因操作系统完善以及便于培养和大规模发酵等优势而作为常用模式生物，并且其内源性的甲羟戊酸途径以及自身麦角固醇与其他中间体甾醇的生物合成相似，更加突出了酿酒酵母作为甾醇及其衍生物的人工合成生物生产底盘细胞的潜力[78]。

早在 1998 年，在酿酒酵母中实现了菜油甾醇、孕烯醇酮及黄体酮的从头合成[79]，又于 2003 年实现了氢化可的松的从头合成，这些工作打开了利用酿酒酵母从简单碳源合成甾醇的大门[80]。随后胆固醇、24-羟基胆固醇等也在酿酒酵母中完成了从头合成[81-82]。近期，Jiang 等[83]在酿酒酵母中实现了油菜素内酯半合成的前体 24-表-麦角甾醇(24-epi-ergosterol)的高效制备，产量达到了约 2.76 g/L，为向市场提供与 24-表-油菜素内酯相似价格的天然油菜素内酯奠定了基础。此外，解脂耶氏酵母等非传统酵母也被开发用于生产甾醇。

为了提高酵母中异源甾醇的产量，国内外学者基于内源调控机制开发了多种代谢工程策略，

诸如加强产物前体供应、过表达限制步骤关键酶、抑制竞争支路的通量强化目标甾醇合成、调控脂滴和细胞膜以此增强甾醇积累和稳态平衡等[84]。一些典型的案例如下：Guo 等[85]筛选 11 个不同来源的 7-脱氢胆固醇(7-dehydrocholesterol, 7-DHC)的合成关键酶 DHCR24 [原鸡(*Gallus gallus*)来源的最优]以及与不同强度启动子偶联，再通过敲除 *ERG5*、*ERG6* 来阻断副产物路径以及调控脂滴相关基因 *NEM1*，将 7-DHC 的产量提高至 1.07 g/L。Qu 等[86]发现弱化调控竞争支路的关键基因 *ERG6* 比敲除更有利于 7-DHC 的生产，其产量提升至 1.328 g/L。Meng 等通过增加 *DHCR7* 的拷贝数同时敲除 *MFE1* 基因提高脂滴的合成来促进菜油甾醇的积累，获得的菌株菜油甾醇产量是出发菌株的 3.7 倍(837 mg/L)[87]。Zhang 等[88]将不同来源间的 CYP11A1 和电子传递配体 Adx/AdR 进行组合以及偶联不同强度的启动子调控表达，最终得到的菌株孕烯醇酮产量达到了 78.0 mg/L。Xu 等[89]筛选薯蓣皂素合成关键的细胞色素 P450 (cytochrome P450, CYP)与细胞色素 P450 还原酶(cytochrome P450 reductase, CPR)的最佳比例(CYPs:CPR=7.5:1)，以及弱化 *ERG6* 基因的表达，最终菌株的薯蓣皂素产量提升至克级水平(2.03 g/L)。尽管如此，异源合成甾醇仍然还存在一些障碍[90]：(1) 异源酶和甾醇前体的供不足。Kim 等[91]通过过表达调节因子 Ino2p 实现了扩展内质网空间以及增强蛋白合成能力，使得 P450 酶的合成以及甾醇合成前体角鲨烯积累显著提升，为解决异源酶和甾醇前体的供应不足问题提供了思路。(2) 关键酶和底物因空间定位不同限制了催化效率。Guo 等[92]研究表明，改变 7-DHC 合成关键酶的亚细胞分布能增加酶之间的反应机会，为提高甾醇产量提供了一个很有潜力的策略。(3) 氧化还原扰动和氧化应激。Wei 等[93]研究表明，过表达 *POS5* 增加 NADPH 的供应以及过表达 *CTT1* 降低胞内活性氧后，7-DHC 摇瓶水平的产量达到了 649.5 mg/L。

在酿酒酵母异源合成甾醇的代谢过程中，甾醇既充当着底物的角色也充当着产物的角色。作为底物，一方面由于甾醇在内质网合成后被转运至别的细胞器发挥作用或贮存，而利用甾醇作为底物的细胞色素 P450 酶多定位于内质网，所以造成了酶与底物存在亚细胞区室隔离，这使催化效率降低，导致目标甾醇产物的合成代谢流减少导致产量不高；另一方面，甾醇可以被外源添加作为目标甾醇产物的前体。作为产物，甾醇积累会对酵母细胞产生不同程度的毒害作用。甾醇转运蛋白可以起到平衡细胞生长与生产以提高目标甾醇产物产量的作用。1996 年 Mahé 等确定了 Pdr5p 和 Snq2p 能介导雌二醇的外排，并且评价了 Pdr5p 和 Snq2p 对调节酵母细胞内雌二醇水平的作用。与野生型水平相比 *PDR5* 单敲除菌株中雌二醇积累增加了约 3 倍，再继续敲除 *SNQ2* 观察到胞内雌二醇积累升高近 10 倍[69]。Chen 等[94]对新月弯孢霉(*Curvularia lunata*) ATCC™ 12017 进行转录组测序，发现了一个转录水平上调了 100 倍的 ABC 转运蛋白 *Cl*Cdr4p，该蛋白由 1 588 个氨基酸组成，与酿酒酵母的 Pdr5p 蛋白和白色念珠菌的 Cdr4p 蛋白序列相似性分别达到 45%和 51%。随后在酿酒酵母中利用 *PGK1* 启动子过表达 *ClCDR4* 构建菌株 HC017，*ClCDR4* 的表达显著促进了底物脱氧可的松 21-醋酸酯(acetylated cortexolone, RSA)向细胞内转运的速率，增加胞内前体的供给促进了后续羟化反应的进行，最终使 HC017 的终产物氢化可的松的产量从 223 mg/(L·d)提高到 268 mg/(L·d)，产率提升了约 20%。Xu 等[89]在酿酒酵母中实现了克级水平(2.03 g/L)薯蓣皂素的从头合成，但仍然存在问题——扫描电镜结果显示与正常细胞相比，DSG

高产菌株细胞 LP118 体积小，且细胞表面的褶皱更多，表明胆固醇或 DSG 的过度积累对酵母细胞的结构和生理产生了不利影响。菌株 LP118 高密度发酵后期出现黑色分泌物质，鉴定后发现是较纯的薯蓣皂素，表明其可以被分泌至胞外。且分析 LP118 转录组数据，发现甾醇转运蛋白 Pdr5p 和 Pry1p 的表达量随着产量的提升而上调，并且同时敲除 *PDR5* 和 *PRY1* 后 DSG 的积累显著降低了 90%，这暗示了甾醇转运蛋白在外排异源甾醇实现增产目标的潜在作用。

在植物体内，甾醇是次级代谢产物，由于皂苷相比于皂素溶解性更好，更容易运输至植物表面组织从而抵御外界微生物侵染，因此植物中皂素多以皂苷形式进行贮存[95-96]，但是具体的转运路径还不清晰。例如薯蓣皂素在细胞质经 UDP-葡萄糖基转移酶 *Dz*S3gtp (3-O-sterol glycosyltransferase)催化作用后薯蓣皂素的 C-3 位置上与 1 分子葡萄糖连接为延龄草素，进而修饰为薯蓣皂苷，更容易被运输到液泡中贮存，不仅增加了其稳定性而且可以保护植物免受次级代谢产物过量导致的毒害作用[97]。此外，能催化麦角固醇、菜油甾醇、β-谷甾醇、豆甾醇、胆固醇和延龄草素等的糖基转移酶也已有报道，因此甾醇的糖基化修饰也是非常重要的转运及贮存的潜在策略[96-100]。

3 总结与展望

甾醇是细胞膜的重要组成成分，也是多种重要细胞代谢物的中间体，其在细胞内质网中完成合成，随后被转运至其他细胞器发挥作用或贮存。酵母中甾醇主要以蛋白质介导的非囊泡运输机制来完成转运，与甾醇转运相关的蛋白包含 OSBP/ORPs 家族蛋白、LAM 家族蛋白、ABC 转运家族蛋白、CAP 超家族蛋白和 NPC 样甾醇转运蛋白。这些蛋白的转运机制不尽相同，不同甾醇转运蛋白功能之间也存在着明显的冗余，但都共同承担起了甾醇在酿酒酵母细胞中转运的工作，保证了酵母胞内甾醇的合理分布与功能稳定。

甾体药物是除抗生素外的第二大药物，具有广阔的市场，合成生物学作为合成甾体药物的绿色途径，越来越被看好。近些年来，酿酒酵母作为宿主异源合成甾醇的工作取得了很大的进展，甾醇的积累得益于许多代谢工程策略的开发，包括过表达限制步骤关键酶、抑制竞争支路的通量、加强产物前体供应和重建辅因子的平衡等，异源酶和甾醇的承载能力低以及区室隔离问题也逐渐得到改善。但随着甾醇产量的不断提升，甾醇的疏水属性以及过多积累引起的细胞毒性，对进一步提高甾醇产量的不利影响变得不能忽视。针对此类问题，可以通过脂滴工程和膜工程来分别增强甾醇酯和游离甾醇在胞内的容纳能力，也可以考虑借鉴植物中的甾醇的解毒贮存机制，以便于进一步解除过量甾醇对酵母细胞的毒副作用。目前，甾醇转运蛋白在酵母细胞工厂中的应用不多，可以针对甾醇转运的特点不断深入研究以加强应用。通过计算机辅助设计转运蛋白已有许多成功案例，但是甾醇转运蛋白相关的设计暂未被报道，随着甾醇转运蛋白晶体结构的解析，未来这将是一个很有潜力的策略；通过分子对接和分子动力学模拟得到蛋白与底物结合的关键位点，再通过定向突变等策略强化其与特定甾醇的结合功能或者拓展其底物的结合范围。同时深入研究蛋白的转运机理，通过添加分泌信号肽等策略强化其转运速率，进一步克服甾醇积累带来的细胞毒性作用，以期进一步提升目标甾醇产物的产量和完善甾醇转运蛋白的研究。

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