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Review 综 述

酵母中葡萄糖阻遏作用机制研究进展

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摘　要：大多数微生物通过一种复杂的机制来感知和传递环境中的葡萄糖变化并对其做出适当的反应。酵母细胞中，葡萄糖主要通过 Snf1/Mig1 信号通路来阻遏三羧酸循环、糖异生、乙醛酸循环和替代碳源代谢等相关基因的转录表达。木糖、半乳糖、蔗糖、乙醇和有机酸等替代碳源只有当环境中的葡萄糖消耗殆尽后才能重启代谢编程，进行替代碳源的利用。而葡萄糖去抑制对于提高现代微生物工业生产效率、解决环境与能源问题具有重要意义。本文综述了 Snf1/Mig1 信号通路阻遏机制以及相关转录因子的活性位点，具体介绍了多种替代碳源的应用以及其受葡萄糖阻遏的具体机制，总结提出了根据不同背景缓解或解除碳代谢阻遏的策略，以期为酵母菌现代化生产应用范围的扩大和效率的提高提供新思路。

关键词：葡萄糖阻遏作用；基因工程；酵母菌；葡萄糖信号通路；混合碳源利用

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Mechanism of glucose repression in yeast

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Abstract: Most microorganisms respond to glucose *via* complicated sensing and signaling mechanisms. In yeast cells, glucose represses the expression of genes involved in the tricarboxylic acid cycle, gluconeogenesis, glyoxylate cycle, and alternative carbon source metabolism mainly through the Snfl/Mig1 signaling pathway. Alternative carbon sources such as xylose, galactose, sucrose, ethanol, and organic acids reset the sugar metabolism only upon the full consumption of glucose in the environment. Derepression of glucose is of great significance for improving the production efficiency of modern microbial industry and addressing environmental and energy issues. This paper introduces the repression mechanism of Snf1/Mig1 signaling pathway and the active sites of related transcription factors. Specifically, we introduce the application of alternative carbon sources and the corresponding mechanisms of glucose repression. Finally, we summarize the solutions to relieving glucose repression on the basis of different backgrounds. This review aims to provide new ideas for expanding the applications and improving the efficiency of modern yeast production.

Keywords: glucose repression; genetic engineering; yeast; glucose signaling pathways; mixed carbon source utilization

在长期适应自然的进化过程中，微生物大多优先使用生长速率最佳、酶利用效率最高、蛋白质合成转化率最高的碳源，因而对不同碳源的利用形成了不同的优先级[1-2]。通常葡萄糖为微生物的首选碳源，当环境中葡萄糖充足时，微生物仅利用葡萄糖而无法代谢其他碳源进行生长；但当葡萄糖缺乏时，微生物也可以利用其他碳源存活下来。这种微生物优先利用葡萄糖且阻遏其他碳源代谢的现象叫做葡萄糖阻遏作用(glucose repression)，也称葡萄糖效应(glucose effect)。

酵母菌为单细胞真核微生物，是工业发酵生产重要的细胞工厂，食品发酵的主要微生物。酵母菌在生长过程中首先会利用环境中的葡萄糖为唯一碳源，只有当葡萄糖消耗殆尽后，酵母菌经历一段时间生长停滞完成替代碳源的代谢重构，才重新开始利用替代碳源进行二次生长[3]。酵母菌的这一特性限制了酵母混合糖利用效率、有机质和酶的生产，以及在食品工业生产中的应用。而酵母葡萄糖阻遏效应的机制复杂，多种阻遏机制协同作用，不同酵母菌种的阻遏机制存在差异，不同代谢基因受到阻遏的机制也并不完全相同，所以对于酵母葡萄糖阻遏机制的研究也复杂而多变。本综述首先讨论了酵母葡萄糖阻遏效应的具体机制，重点介绍了不同替代碳源受葡萄糖阻遏的机制解析，最后总结了缓解或解除葡萄糖阻遏作用的方法，以期更加全面客观认识和了解葡萄糖效应。

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1 酵母葡萄糖阻遏作用机制

1.1 Snf1/Mig1 通路阻遏机制

酵母碳分解代谢物阻遏机制是目前研究最清楚的葡萄糖信号通路之一。酵母中调控葡萄糖感应和变化的信号通路整合于一个调节网络中，以确保葡萄糖的有效摄取和利用[4]。其中 Snfl/Mig1 信号通路主要受葡萄糖调控，负责调节碳分解代谢物抑制[5]。

如图 1 所示，葡萄糖通过 Snf1/Mig1 通路于酵母细胞信号系统中层级传递环境中葡萄糖浓度信号，并最终调控相关基因的表达。当环境中没有葡萄糖时，Mig1 处于蛋白磷酸化状态，定位于酵母细胞质中；当加入葡萄糖后，Mig1 去磷酸化，定位于酵母细胞核中，并通过与下游靶基因启动子结合行使阻遏功能。Mig1 磷酸基团转移机制主要受到 Snf1 的调控。在高浓度葡萄糖中，Snf1 去磷酸化而失去激酶活性，此时 Snf1 无法对 Mig1 进行磷酸化，使得 Mig1 定位于细胞核中，招募阻遏复合物 Cyc8/Ssn6-Tupl 与被抑制的靶基因的启动子区域结合[6-7]，发挥阻遏作用下调相关基因的表达；随着葡萄糖的耗尽，Snf1 重新磷酸化并开始发挥功能，进而磷酸化 Mig1，致使 Mig1 由细胞核转运至细胞质，受阻遏基因重新开始转录[5,8-9]。

除了 Mig1 直接作用于相关代谢基因的启动子外，Snf1/Mig1 机制还涉及转录因子的层级调控机制[10-11]。CAT8、GAL4、ADR1、SIP4、HAP 复合物等不可发酵碳源、糖异生、三羧酸循环、呼吸和乙醛酸循环相关基因的转录激活因子同样受到 Snf1 与 Mig1 的调控[12-18](表 1)。葡萄糖存在环境下，去磷酸化 Snf1 激酶失去对 CAT8

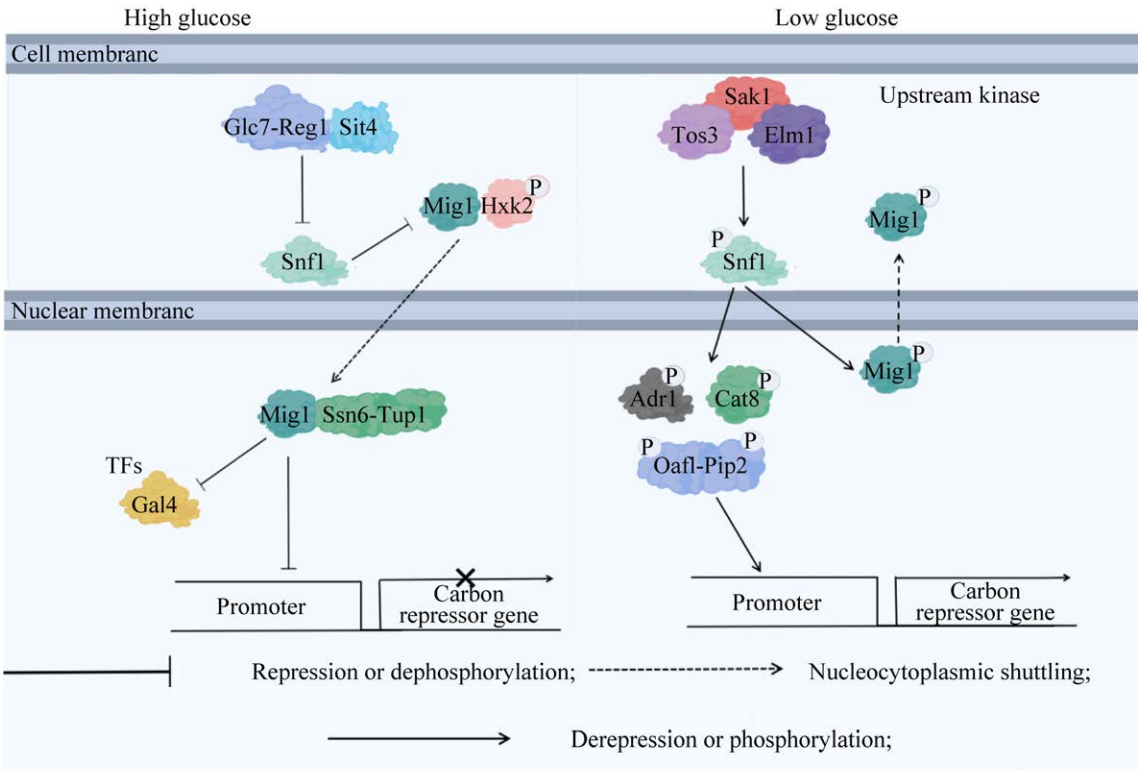

图 1 Snf1/Mig1 信号通路示意图

Figure 1 Molecular mechanism of the signal path Snf1/Mig1.

转录因子功能激活作用[19-20]，同时也有证据表明 Mig1 直接对 CAT8 的转录表达起抑制作用[21]，这些均使 CAT8 在葡萄糖存在时无法行使其对下游靶基因的转录激活作用。如环境中葡萄糖消耗殆尽后，Snf1 磷酸化激活 CAT8 转录因子活性，进一步调控 SIP4 的表达，最终激活下游糖异生相关基因表达的重启[22]；Ricci-Tam 等[23]的最新研究结果表明，敲除半乳糖代谢相应转录激活因子 GAL4 基因启动子上报道的 Mig1 结合位点可以部分缓解葡萄糖对半乳糖代谢的阻遏，而同样敲除半乳糖代谢基因 GAL 启动子上的 Mig1 结合位点并不能达到解阻遏的目的，表明葡萄糖浓度介导阻遏因子 Mig1 转录调节 GAL4 的表达水平，进而调控半乳糖代谢基因的转录水平，呈现分级调控模式，进一步验证了葡萄糖阻遏效应的层级调控机制。

表 1　替代碳源的主要转录调控因子及其靶点

Table 1　Major transcriptional regulators of alternative carbon sources and their targets

Activating transcription factor (ATF)	Function	Downstream target genes
GAL4	Activates the expression of genes related to galactose metabolism	GAL1, GAL2[23-24]
ADR1	Activates the expression of genes related to ethanol, glycerol and fatty acid metabolism	ADH2, ACS1, GUT1, POX1[12,17-18]
CAT8	Activates and regulates the expression of genes related to ethanol, lactic acid, glyoxynic acid cycle and gluconeogenesis metabolism	JEN1, ICL1, MLS1, FBP1, PCK1, SIP4[12,17-18,25-26]
SIP4	One of the downstream targets of CAT8, activating and regulating the expression of genes related to gluconeogenesis	FBP1, PCK1[22]
HAP complexes	Activates and regulates the expression of genes related to respiratory metabolism and tricarboxylic acid cycle	CYC1, CIT1, SDH2[16,27]
Oaf1-Pip2	Activates and regulates the expression of genes related to fatty acid metabolism	POX1, POX2[28]

己糖激酶在 Snf1/Mig1 阻遏机制中起重要的信号传导作用。己糖激酶具有双重亚细胞定位：它在细胞质中是一种糖酵解酶，而在细胞核中是作为葡萄糖抑制信号的重要调节因子[29-30]。此外，目前的研究表明，己糖激酶(hexokinase, Hxk)也参与了 Mig1 的定位，调控细胞核内的基因转录。Hxk2 在细胞核和细胞质之间的来回穿梭受到 Ser^{14} 的磷酸化和去磷酸化的调控，且 Snf1 与 Hxk2 的核质转运密切相关[31]。Ahuatzi 等[32]证实 Hxk2 的 Lys_6-Met_{15} 区域是该蛋白核定位以及与 Mig1 蛋白相互作用所必需的。Schmidt 等[33]表明 Hxk1 和 Hxk2 对 Mig1 持续稳定定位于细胞核中发挥了重要作用。Vega 等[34]发现 Hxk2 根据环境中葡萄糖的浓度进行构象转换，在高糖条件下，Hxk2 形成一种紧密的构象，通过促进转录阻遏因子 Mig1 对靶基因的结合实现阻遏效应。目前的研究已证明己糖激酶对 Mig1 发挥阻遏功能有重要协同作用，而己糖激酶构象变化机制以及其通过何种相互作用机制协同 Mig1 行使功能仍存在研究空间。

1.2　Snf1 蛋白激酶

蔗糖非发酵 1 蛋白(sucrose nonfermenting 1 protein, Snf1)蛋白激酶，又叫 CCR1 或 CAT1，处于酵母细胞葡萄糖阻遏调节机制上游的位置，

与哺乳动物中 AMPK 蛋白激酶同源，是酵母细胞能量稳态的主要调节因子，通过磷酸化细胞内相关蛋白协调细胞能量平衡以及调节对葡萄糖匮乏环境的适应[35]。Snf1 对于葡萄糖衰竭后和葡萄糖抑制结构基因去抑制之前发生的代谢开关至关重要，是适应替代底物所必需的[36]。酿酒酵母 Snf1 激酶是由 α 催化亚基(Snf1)、核苷酸结合 γ 亚基(Snf4)和 3 个替代 β 亚基(Gal83、Sip1 或 Sip2)之一组成的异质三聚体[37]。在高糖条件下，Snf1 激酶自抑制域 α 亚基独立存在，但它的活性位点处于封闭状态和非活性构象；在低糖条件下，γ 亚基结合腺嘌呤核苷酸后与 α 亚基结合[38]，催化 α 亚基 Leu^{183} 位点磷酸化，促进其活性位点的开放[39]。Thr^{210} 位点是目前 Snf1 激活环上研究最为深入的磷酸化位点[40-41]，该位点的磷酸化主要由 Sak1 (Pak1)、Tos3、Elm1 催化，其中 Sak1 起主要作用[42-44]，并由 Glc7 与 Reg1 组成的复合物 PP1 磷酸酶以及 Sit4 去磷酸化[45-46]。总而言之，Snf1 激活环磷酸化机制独立于异质三聚体调控[46]，但异质三聚体结构对维持 Snf1 构象稳定和激活环的磷酸化状态至关重要。

1.3 转录阻遏因子

Mig1 是酵母中根据碳源种类和浓度调节磷酸化状态、位于 Snf1 蛋白激酶下游的一种葡萄糖效应转录阻遏因子。其包含 2 个 Cys_2-His_2 锌指结构，2 个锌指结构域能够与许多受葡萄糖阻遏的代谢基因启动子区域结合，通过抑制转录进程发挥阻遏作用[47-48]。Mig1 下游基因启动子上结合位点具有明显的序列特征，由富含 GC 的核心区域(C/G)(C/T)GG(G/AG)G 组成[49]，GC-box 5′端一段富含 AT 的区域也是 Mig1 结合所必须的[50]。低糖条件下 Snf1 催化 Mig1 中 4 个目前已知的 Ser^{222}、Ser^{278}、Ser^{311} 和 Ser^{381} 位点磷酸化[8,51]，此时 Mig1 定位于细胞质中，阻遏作用解除[52]。除此之外酵母中常见的含有锌指结构的转录阻遏因子还有 Mig2[53]、Mig3[54]和 Nrg1[55]。Mig2 通常不单独发挥作用，而是协同 Mig1 进行葡萄糖阻遏的微调，且 Mig2 的作用范围远小于 Mig1[56]。而现有的证据表明，Mig3 与 Mig1 和 Mig2 在功能上并无关系[53]。而目前的研究表明，Nrg1 也是酿酒酵母中重要的转录阻遏因子之一。Zhou 等和 Wang 等的实验均已证明 Nrg1 在高糖条件下阻遏蔗糖、半乳糖以及甘油、甲醇的代谢，而 Nrg1 随葡萄糖调控的作用机制尚不明确，可能与 Snf1-Mig1 通路无关[55-57]。综上所述，Mig1 是酵母中最重要、作用范围最广的转录阻遏因子，而 Nrg1 在对部分碳源的阻遏过程中也起到重要作用，且仍有更多的功能尚待验证。

2 不同碳源代谢进程中的阻遏效应

酵母碳水化合物代谢涉及复杂的调节机制。在葡萄糖存在的情况下，糖的发酵优先于有氧代谢[58]。酵母有氧代谢会增加生物量、有机酸和甘油等副产物的积累[59]，而葡萄糖的存在下调三羧酸循环、糖异生、乙醛酸循环[25,60]、呼吸[61]和氧化磷酸化等中心碳代谢相关通路基因的表达，从而使碳通量主要流向乙醇的发酵生产，以达到更高的乙醇产量，但同时副产物的生产效率降低[62]。因而呈现解阻遏性状的酵母菌株将会进行中心碳代谢通量的重构，中心碳代谢通量的重编程对葡萄糖消耗结束后多种碳源发酵的重利用有重要作用。

酵母中葡萄糖效应阻遏的基因涉及范围广[63]，主要分为 3 种功能。第一类被阻遏基因主要包括三羧酸循环和氧化磷酸化所需的基因，保证葡萄糖的碳通量主要流向乙醇；第二类受到阻遏的基因包括转运和代谢其他碳源所需的基

因，如半乳糖、麦芽糖和乙醇等，以提高葡萄糖的代谢效率和产物产量；第三类葡萄糖阻遏的是参与糖异生的相关基因，它需要阻止葡萄糖的二次合成，以防止无效的循环。尽管不同的代谢通路涉及基因的种类与数量不同，但代谢状态相似的碳源受葡萄糖调控的机制仍有许多相似之处。

2.1 可发酵糖类物质

2.1.1 戊糖

随着环境与能源问题日益严重，利用植物生物质木质纤维素为原料生产第二代燃料和化学产品成为目前的研究热点。木质纤维素中可发酵糖主要为葡萄糖、木糖和 L-阿拉伯糖，其中丰度最高的戊糖为木糖，约占 30%；L-阿拉伯糖的占比约为 1.50%–2.75%[64]。然而葡萄糖抑制限制了木质纤维素原料中混合糖的同时利用[65]，开发能够同时从生物质水解物中发酵混合糖的微生物平台，对于提高规模化工业生产效率及利润至关重要[66]。

酿酒酵母缺少戊糖代谢的初始途径，目前工业上通常通过引入外源基因获得戊糖代谢能力的重组酿酒酵母菌株[66-67]。酿酒酵母混合糖代谢过程中，葡萄糖对戊糖摄取转运有明显的抑制作用为戊糖代谢受阻的主要原因，同时泛素化降解戊糖相关转运蛋白[68]，即酿酒酵母缺乏特异性识别戊糖和重新编程细胞代谢转化为戊糖利用状态的信号通路[69]。酿酒酵母缺乏特异性木糖和阿拉伯糖转运系统，常见的酿酒酵母戊糖转运体 HXT7、HXT5、GAL2、HXT1、HXT4、HXT36 同样可以识别葡萄糖，且对葡萄糖的亲和力远高于戊糖[70]。构成性表达经过分子改造后、具有高木糖亲和力的戊糖转运体如 GAL2、HXT7 和 HXT36[71-73]，以及表达其他真菌特异性戊糖转运蛋白[74-75]，以减轻葡萄糖对戊糖代谢摄取的抑制。Subtil 等[76]认为葡萄糖的分解代谢也会阻碍戊糖的利用，这可能与 Snf1 和己糖激酶的调控有关[77]，且部分戊糖转运蛋白如 GAL2 转录也会受到葡萄糖调控。而部分天然具有戊糖代谢能力的非酿酒酵母菌株由于 Snf1/Mig1 通路尤其是己糖激酶对木糖代谢关键基因木糖醇脱氢酶基因的抑制，阻碍了混合糖的共利用[78-79]。同时由于葡萄糖利用结束后在进行戊糖的二次发酵时，戊糖利用能力较单一戊糖纯培养时显著下降，关于葡萄糖后继效应的研究也为提高混合糖发酵性能提供了思路[80-81]。

2.1.2 己糖

通常初始葡萄汁含有等量的葡萄糖和果糖，但酵母对葡萄糖的发酵速度略快于果糖，导致发酵后期果糖浓度高于葡萄糖浓度，因而酵母必须在饥饿和大量乙醇存在的情况下发酵这种非首选糖源。在葡萄酒领域，高果糖/葡萄糖比值可能导致发酵缓慢、停滞以及葡萄酒的不良甜味，这是全球葡萄酒行业的一个主要问题[82]。但该现象产生的原因并不是葡萄糖信号分子转录层面的阻遏，而是相较于果糖，葡萄糖与部分己糖激酶和己糖转运蛋白的亲和力更高有关[83-84]。

葡萄糖对半乳糖的糖阻遏作用是研究最深入的真核生物信号整合系统之一。GAL4 是启动 GAL1、GAL2、GAL3、GAL80 等半乳糖代谢基因转录的转录因子[24]。GAL1 编码半乳糖激酶，于半乳糖分解代谢的第一步磷酸化半乳糖。GAL2 是一种位于细胞膜上的半乳糖渗透酶，对半乳糖和葡萄糖均具有高亲和力，GAL2 基因的表达由半乳糖诱导，并受葡萄糖阻遏[85]。相反，GAL80 是一个负反馈节点，隔离 GAL4，阻断 GAL 代谢基因的转录[86]。GAL3 是一种细胞内半乳糖传感器，与半乳糖结合后被激活[87]。活化的 GAL3 与 GAL80 形成复合物，缓解 GAL80 对 GAL4 的抑制。Duan 等[88]从马奶酒自然生境中筛选出逆向进化的酿酒酵母菌株通过半乳糖代谢网络调控元件的一系列协同变异，完全解除

葡萄糖阻遏效应，能够快速、优先利用半乳糖，同时维持代谢葡萄糖的能力，为构建同时高效利用不同碳源的酵母工程菌提供了新的策略。过去的研究者认为葡萄糖的浓度到达一定阈值时，酵母细胞即会响应葡萄糖阻遏效应，抑制半乳糖的代谢，而 Escalante-Chong 等[89]的实验证明半乳糖对葡萄糖抑制的响应是根据环境中半乳糖/葡萄糖浓度的比例决定，并提出半乳糖/葡萄糖的比例，决定了细胞是否响应葡萄糖阻遏，而葡萄糖浓度独立地决定对下游基因阻遏强度的新模型[23]。高浓度葡萄糖条件下 Snf1/Mig1 通路通过抑制半乳糖代谢途径中的多个基因行使阻遏功能[90]，而也有研究认为 Snf1/Mig1 通路主要通过抑制对半乳糖代谢起整体调节作用的转录因子 GAL4 的表达，从而达到对半乳糖代谢的全局阻遏[23]。

2.1.3 二糖、三糖和多糖

除麦芽糖通过特殊的渗透酶转运进入酵母细胞中后分解[91]，其他二糖、三糖和多糖需要在细胞膜外被水解成单糖，才能被酵母细胞吸收。葡萄糖是大多数二糖、三糖、多糖水解的产物之一，由于水解后的葡萄糖的广泛存在，使得葡萄糖阻遏效应严重阻碍了它们的水解效率。酵母对蔗糖[92-93]、乳糖[94-95]和麦芽糖[96-97]等二糖的代谢均存在葡萄糖阻遏效应，其阻遏机制均与 Snf1/Mig1 通路关系密切，其中蔗糖代谢基因 SUC2 是其中研究最多的基因之一。Lutfiyya 等[56]通过研究 Mig1、Mig2 与 SUC2 基因启动子的结合发现，Mig1 和 Mig2 结合到其启动子上相似的位点，进而对 SUC2 的表达起到阻遏作用，从而调控蔗糖代谢。另外，针对乳糖利用的研究中，半乳糖苷酶能将乳糖分解为葡萄糖和半乳糖。Zhou 等[94]将马克思克鲁维酵母 Mig1 基因进行敲除，发现其缺失菌株半乳糖苷酶的产生和半乳糖苷酶基因的表达较野生型菌株提高了 2 倍左右，能有效水解不同来源的乳糖。酵母麦芽糖代谢与啤酒发酵和面包制作密切相关，而葡萄糖效应严重阻碍了麦芽糖代谢的速率。麦芽糖首先通过麦芽糖渗透酶转运至细胞，然后被麦芽糖酶水解成 2 个单位的葡萄糖。而麦芽糖渗透酶和代谢酶基因的转录均受到 Snf1-Mig1 通路的阻遏[98-99]。另外，部分非酿酒酵母具有分泌棉子糖、菊粉水解酶的能力，但其水解酶的受到葡萄糖浓度的调节。Lertwattanasakul 等[100]发现马克思克鲁维酵母中，高温可以部分缓解葡萄糖对菊粉、棉子糖水解的抑制。Georis 等[101]证实乳酸克鲁维酵母菊粉水解酶(INV1)基因转录受到强烈葡萄糖抑制，但其抑制机制与 Mig1 无关，仍需要更多的研究以探索和完善非酿酒酵母中葡萄糖对三糖以及多糖代谢的抑制机制。

2.2 不可发酵型碳源

2.2.1 醇类物质

ADR1 和 CAT8 是酵母中受营养调节的转录激活因子，它们在没有可发酵碳源的情况下，共激活非发酵碳源如乙醇和甘油代谢所必需的基因。研究表明，ADR1 是激活调控乙醇代谢相关基因 ADH2、ACS1、甘油代谢相关基因 GUT1 和表达所需的转录激活因子[17-18]。Young 等[14]通过转录组分析表明，在 ADR1 缺失的情况下，108 个基因的表达显著降低，而其中大多数参与了不同非发酵碳源的氧化，对于乙醇、甘油、乳酸和脂肪酸氧化形成乙酰辅酶 a 的过程起重要作用，并产生大量 NADH，且没有一个 ADR1 依赖的基因在以葡萄糖为碳源的培养基中生长是必需的。同时对 ΔADR1 和 ΔCAT8 重组菌株的转录组聚类分析以及免疫共沉淀分析表明，有少量的 ADR1 依赖的基因也依赖于 CAT8，一些编码代谢乙醇和乳酸的酶的基因共同依赖于 ADR1 和 CAT8 来解除阻遏作用[14,102]。

大多数研究认为 ADR1 的表达与 Snf1 的磷

酸化状态有关，当葡萄糖存在时，Snf1 处于非磷酸化状态，ADR1 无法得到磷酸化激活，非发酵碳源相关基因的表达下调；而当环境中的葡萄糖消耗殆尽后，Snf1 重新磷酸化并激活 ADR1 与下游靶基因结合，启动非发酵碳源相关基因的表达[14]，然而其中的调节机制仍存在争议，目前并没有证据表明 Snf1 与 ADR1 有直接相互作用。有研究表明 Snf1 主要在染色质结合水平而不是转录激活水平调控 ADR1 对下游靶基因的激活，促进 ADR1 在高乙酰化组蛋白存在的抑制条件下与染色质的结合[103]。Ratnakumar 等[104]研究表明 Snf1 通过间接调控 ADR1 Ser^{230} 位点去磷酸化而改变 ADR1 的活性，但并未找到 ADR1 的特异性作用因子。ADR1 的调控可能还与 14-3-3 蛋白 BMH 有关。Young 等[105-106]的研究团队发现 BMH 与 ADR1 启动子结合并抑制其转录活性，在葡萄糖存在和不存在的情况下，BMH 活性的降低均会导致 ADR1 下游基因表达水平升高，并通过共免疫沉淀实验结果证实二者之间确实存在相互作用。CAT8 是在非发酵碳源上生长的必要基因，如前文所述，大多数编码乙醛酸循环酶和糖异生的 2 个关键酶的基因都是 CAT8 的良好特征靶点，而非发酵碳源的代谢与这些途径有密切的联系[13]。

除大多数酵母均可代谢的乙醇、甘油外，毕赤酵母依赖甲醇氧化酶基因 AOX1 代谢甲醇。毕赤酵母表达系统外源蛋白表达量高，遗传稳定，培养成本低且产物易分离，主要应用可受甲醇严格调控的 AOX1 启动子表达外源蛋白。但 AOX1 的表达只受甲醇诱导，并且受到葡萄糖、乙醇、甘油等其他碳源的强烈抑制[107]。现有的研究表明，优势碳源对 AOX1 基因的阻遏与 Mig1 等转录阻遏因子功能相关。Prielhofer 等[108]发现毕赤酵母对不同碳源的响应依赖转录水平而不是翻译水平调节。张平[109]发现 Mig2 不单独对 AOX1 基因起阻遏效应，但毕赤酵母 Mig1 和 Mig2 双缺失菌株的解阻遏效果高于 Mig1 单缺失菌株。Wang 等[57]发现在葡萄糖和甘油中毕赤酵母转录因子 Nrg1 同样抑制 AOX1 基因的表达，Nrg1 通过和该基因转录激活因子 Mxr1、Prm1 竞争启动子上的结合位点发挥阻遏作用，并且得出 3 个抑制因子的抑制强度排序为 Mig1>Nrg1>Mig2。Zhan 等[110]发现甘油对 AOX1 表达的下调可能与甘油转运体有关。缓解其他碳源对 AOX1 启动子的抑制对毕赤酵母外源基因表达效率的提高具有重要的意义。

2.2.2 脂肪酸

酿酒酵母可以代谢油酸，但为了防止代谢的无效循环，脂肪酸 β-氧化相关的基因受到葡萄糖阻遏效应的抑制。油酸代谢相关的过氧化物酶体功能基因的转录激活受到 OAF1、PIP2 和 ADR1 转录因子的调控[111-112]。OAF1 和 PIP2 以异源二聚体的形式与靶基因的油酸响应元件结合，在葡萄糖去抑制及油酸诱导条件下发挥作用[28]。与上述非发酵碳源的调节机制类似，Snf1 是葡萄糖消耗殆尽后激活上述转录因子所必需的激酶，其借助转录因子 ADR1 与 OAF1 和 PIP2 相互作用调控酵母中过氧化物酶体增殖和氧化功能[113]，但目前尚无 Snf1 直接作用于 OAF1-PIP2 的证据。

2.2.3 有机酸

目前已有研究发现，环境中葡萄糖的存在抑制酿酒酵母对丙酮酸、乳酸等的利用。1987 年 Cássio 等[114]的研究提出酿酒酵母在葡萄糖和乳酸为碳源的培养基中先利用葡萄糖再利用乳酸，并认为这一现象产生的原因与乳酸转运体被抑制有关。之后的研究表明葡萄糖抑制酿酒酵母乳酸和丙酮酸转运蛋白 JEN1 基因的转录[115-116]以及加速 JEN1 mRNA 的降解[117]，并且醋酸转运蛋白 ADY2 的基因表达同样受到葡萄糖的抑

制[118]。Tsuboi 等[119]利用葡萄糖类似物 2-脱氧-D 葡萄糖培养基筛选出显著提高葡萄糖和丙酮酸共代谢能力的突变酿酒酵母菌株，应用该菌株进行清酒发酵显著改变了成酒中有机酸组成。Balderas-Hernández 等[120]通过敲除酿酒酵母 *Mig1* 基因发现，突变菌株在乙酸、甲酸和乙酰丙酸毒害浓度下的耐受性和发酵性能得到显著提高，同时该菌株可以在厌氧和好氧的条件下共同代谢葡萄糖和甲酸。总而言之，现有的研究证明葡萄糖存在时 Snf1/Mig1 通路直接或间接通过转录因子 CAT8 抑制羧酸转运蛋白基因的表达[26]，同时加速其 mRNA 的降解，但 mRNA 降解的机制仍然存在争议。

利用降酸非酵母进行高酸果酒发酵以调节成酒口感平衡的方法是果酒产业的目前的研究热点，而葡萄糖对苹果酸、柠檬酸等多元酸代谢的抑制是果酒生物降酸发展的瓶颈之一。乳酸克鲁维酵母(*Kluyveromyces lactis*)中发现 2 个 JEN1 同源物可分别转运一元羧酸及二元羧酸，同时基因表达受到葡萄糖抑制[121]。发酵毕赤酵母(*Pichia fermentans*)、季也蒙毕赤酵母(*Meyerozyma guilliermondii*)和毕赤酵母(*Pichia pastoris*)中发现葡萄糖的存在抑制菌株对苹果酸和柠檬酸的利用，导致有机酸的总利用率降低[122]。毕赤酵母 *Mig1* 与 *Mig2* 双缺失菌株与野生型菌株的转录组分析显示，重组菌株柠檬酸代谢途径相关基因上调，说明柠檬酸代谢受到抑制与 Snf1/Mig1 通路可能有关[123]。目前关于葡萄糖对有机酸代谢层面阻遏机理的相关研究较少，结合实验室前期研究成果表明，其可能与葡萄糖效应对酵母细胞羧酸转运蛋白、糖异生、乙醛酸循环、三羧酸循环和呼吸链等途径相关基因的阻遏有关，而柠檬酸代谢受到阻遏还可能与葡萄糖下调柠檬酸裂解酶 ACL 基因表达有关[124](表 2)。

3 缓解或解除葡萄糖阻遏作用的策略

3.1 基因工程改造

3.1.1 转运体进化

酿酒酵母部分可发酵碳源的转运体基因表达受到葡萄糖的抑制，或者像木糖、果糖等与葡萄糖共用同一个转运体，但转运体对葡萄糖的亲和力远高于替代碳源，通过鉴定和替换替代碳源专一性转运体、应用分子操作提高转运体对替代碳源的亲和力均可以缓解葡萄糖对替代碳源代谢的抑制作用。Jiang 等[74]从可以利用木糖的丝状真菌中鉴定出新的木糖转运体 XLTR1，并对其进行分子改造后表达至酿酒酵母中，达到高效转运木糖而不转运葡萄糖的目的。Reznicek 等[133]运用易错 PCR 及流式细胞仪高通量筛选技术得到 8 个与野生型相比葡萄糖亲和力降低而木糖摄取能力相对提高的 GAL2 突变体，通过对不同突变体突变位点的分析研究以确定基因工程改善混合糖吸收的关键靶点。

3.1.2 转录阻遏因子基因敲除

基因敲除是最简单直接改变微生物性状的方法之一。通过敲除葡萄糖阻遏机制通路上的关键基因是缓解或解除葡萄糖效应最常用的方法。Lin 等[99]通过对面包酵母野生型与 Mig1、TUP1、SSN6 三个葡萄糖阻遏相关基因的单缺失突变株、双缺失和三缺失组合突变株共代谢麦芽糖与葡萄糖能力的比较，发现 *Mig1* 和 *SSN6* 单缺失和双缺失菌株解阻遏效果显著，成功提高面包酵母分解麦芽糖的效率；酿酒酵母ΔMig1 重组菌株与野生型菌株相比，重组菌株葡萄糖抑制作用明显减弱，在需氧条件下具有较高的生长效率，代谢状态由发酵部分转向有氧代谢，导致如乙醇和醋酸盐等发酵产物减少，同时有氧代谢产物如甘油和丙酮酸等含量增加[62]。

表 2　葡萄糖效应阻遏替代碳源代谢机制

Table 2　The metabolic mechanism of carbon catabolite repression

Type of carbon source			Species	Major mechanisms or genes	Cases of relieving carbon catabolite repression
Fermentable carbon source	Monosaccharide	Pentose	*Saccharomyces cerevisiae*[77]	Transporter competition inhibition[70]	Adaptive evolution[78,125]
			Kluyveromyces marxianus[79,125]	XDH[78-79]	Transporter evolution[71-75]
			Scheffersomyces stipitis[78]		
		Fructose	*Saccharomyces cerevisiae*[82]	Transporter[84,126], Hexokinase competition inhibition[83]	Adaptive evolution[127] Transporter evolution[128]
		Galactose	*Saccharomyces cerevisiae*[89]	GAL4, GAL1[23,89]	Mig1 expression regulation[129] Mutation in downstream binding sites of transcriptional repressors[23]
	Disaccharide	Sucrose	*Saccharomyces cerevisiae*[92-93]	SUC2[56]	Mig1 expression regulation[92] Exogenous addition of inositol[130-132]
		Lactose	*Kluyveromyces marxianus*[94]	β-galactosidase gene[94]	Mig1 expression regulation[94]
		Maltose	*Saccharomyces cerevisiae*[96]	MAL61, MAL62, MAL63[97-98]	Mig1 expression regulation[96-97,99]
	Polysaccharide	Inulin	*Kluyveromyces marxianus*[100]	INU1[101]	
			Kluyveromyces lactis[101]		
Non-fermentable carbon source	Alcohols	Glycerol	*Saccharomyces cerevisiae*[14,17-18]	GUT1[14,17-18]	
		Ethanol	*Saccharomyces cerevisiae*[14,17-18]	ADH2, ACS1[14,17-18,102]	
		Methanol	*Pichia pastoris*[107]	AOX1[107]	Mig1 expression regulation[107,109]
	Fatty acid	Oleic acid	*Saccharomyces cerevisiae*[28,111-113]	POX1, FOX3, PEX11[28,111-113]	
	Organic acids	Formic acid	*Saccharomyces cerevisiae*[120]		Mig1 expression regulation[120]
		Pyruvic acid	*Saccharomyces cerevisiae*[119]	JEN1[119]	Mutagenesis screening[119]
		Citric acid	*Kluyveromyces lactis*[121]	JEN1[114-115], ACL[124], TCA cycle	
		Malic acid	*Pichia fermentans*		
			Meyerozyma guilliermondii		
			Pichia pastoris[122]		
		Lactic acid	*Saccharomyces cerevisiae*[114]	JEN1[114]	

3.1.3 突变 Snf1/Mig1 通路作用因子磷酸化状态

Snf1 蛋白激酶处于酵母细胞葡萄糖阻遏调节机制的上游，通过磷酸化细胞内相关转录因子，实现胞内能量平衡以及适应葡萄糖匮乏环境，调节 Snf1 及与其相互作用的 Mig1、Hxk2、Sak1、Glc7 等作用因子磷酸化状态可能在解除葡萄糖阻遏效应中具有重要作用。目前已有研究发现提高 Snf1 磷酸化水平，可以部分解除葡萄糖对羧酸转运蛋白 JEN1 表达的抑制，从而显著提高葡萄糖和丙酮酸共代谢能力[119]。而 Snf1 和 Mig1 等蛋白的磷酸化位点已明晰，可通过定点突变、基因编辑等技术调节相关作用因子的磷酸化水平。

3.1.4 生长素诱导 Snf1/Mig1 通路作用因子降解

生长素诱导蛋白降解(auxin induces protein degradation, AID)系统是一种高效降解多种靶蛋白的技术。该系统通过向酵母细胞中引入植物生长素受体 F-box 蛋白，触发与 AID 序列标记靶蛋白的相互作用，最终导致靶蛋白的泛素化降解。Lu 等[134]通过在酿酒酵母中构建 Hxk2 生长素诱导降解系统，实现了细胞中 Hxk2 的降解，部分解除了葡萄糖阻遏，成功将橙花醇的产量提升至 3.4 g/L。Hayat 等[129]于酿酒酵母中引入 Mig1 生长素诱导降解系统，以解除葡萄糖对 GAL 启动子表达的抑制，橙花醇的产量相较于初始提高了 2 倍，证明了该系统在解除葡萄糖阻遏方面的巨大潜力。

3.1.5 改变 Mig1 核质定位

Snf1 介导 Mig1 去磷酸化后，Mig1 进入细胞核内，通过与非优势碳源分解代谢相关基因的启动子结合，阻遏相关碳源的分解代谢。研究表明，通过突变特定调控因子的潜在磷酸化位点或其核定位序列和定位调控序列，或者利用雷帕霉素介导的 FK506 结合蛋白-雷帕霉素结合域(FK506 binding proteins (FKBP)-rapamycin binding domain, FRB)之间的二聚化作用，可调控转录因子向核内、外的转运。张伟平[135]通过构建雷帕霉素介导的蛋白锚定系统改变了氮分解代谢阻遏因子 Gzf3 和 Dal80 的核质定位，并据此阐明了 Gzf3 和 Dal80 在氮分解代谢阻遏效应中向下游传递信号的方式。

3.1.6 突变转录阻遏因子下游结合位点

Mig1 与受葡萄糖阻遏的基因启动子特定区域结合，行使转录阻遏功能。敲除相关基因启动子上的 Mig1 结合位点，可有效缓解葡萄糖阻遏效应。Ricci-Tam 等[23]通过突变半乳糖代谢基因转录激活因子 GAL4 基因启动子上与 Mig1 的结合位点，增强了葡萄糖存在时 GAL4 基因的表达水平，从而解除了葡萄糖对半乳糖代谢基因 GAL 的表达抑制。该方法构建的菌株只针对特定性状的解阻遏，而对整体代谢影响较小，应用于实际生产时突变菌株仍可行使正常的发酵功能和对其他碳源的阻遏作用。

3.2 适应性进化

适应性进化能够短时间内有效改变菌株的某些生理特性，并且基本不会影响除目的性状外的其他优良特性，是菌种改良的重要方法之一。Papapetridis 等[77]将实验室构建的重组木糖菌株于葡萄糖-木糖混合物上进行连续分批培养，获得了能够快速共同消耗 2 种糖的突变体，并对其进行全基因组测序，将突变基因引入非进化的木糖发酵酿酒酵母菌株，得到一株木糖和葡萄糖共消耗比率比其亲本菌株高 2.5 倍的工程菌株。Nijland 等[72]通过适应性进化得到一株己糖转运体 HXT36-N367A 突变的酿酒酵母菌株，该突变菌株存在葡萄糖转运缺陷同时以较高的速率吸收木糖，使突变菌株葡萄糖和木糖能够进行共同代谢。

3.3 培养基质中添加外源物质

de Souza 等[136]通过转录组测序发现，外源

添加 Mg^{2+}可以部分缓解环境中葡萄糖对酿酒酵母的抑制效应，释放参与氧化代谢、呼吸、糖异生和应激反应相关的基因。培养基中添加足量的肌醇可以缓解葡萄糖抑制，Chi 等[130]于培养基中添加 100 μg/mL 肌醇即可在 20 g/L 葡萄糖条件下部分解除对酵母蔗糖酶分泌的阻遏；培养粟酒裂殖酵母时添加 800 μg/mL 肌醇可以得到相同的结果[131]。方燕[132]发现肌醇的解阻遏作用主要是因为肌醇是酵母细胞中具有第二信使的功能，其通过信号传导介导 Snf1/Mig1 通路来调控蔗糖酶的分泌。这些结果均说明通过添加外源物质引起酵母细胞中信号分子状态变化可能是缓解或解除葡萄糖阻遏的方法之一。

4 结语与展望

葡萄糖效应是自然界生物为了提高代谢效率、长期自然选择形成的一种生理效应，而在科技日益发展、生物资源充分开发和利用的今天，葡萄糖效应给新能源、食品等行业的现代化生产带来了新的困扰。二十世纪以来，研究人员对于半乳糖、蔗糖和中心碳代谢等常见替代碳源和代谢途径的研究已较为全面，而对于木糖、油酸等新能源应用以及有机酸等食品发酵课题的研究尚处于充分发展阶段。而对于葡萄糖阻遏作用的生理机制复杂多变，不同酵母菌种中存在不同程度的差异，还需要进一步对现有研究结果进行完善以及发现更多相关的效应因子和作用机制。首先用于应用的葡萄糖阻遏效应解除菌株仍要考虑对菌株其他发酵性能的影响，部分碳源涉及的被抑制基因广泛或者与葡萄糖等多条代谢途径关系密切，不合理的去阻遏方式容易造成菌株其他优良性状的破坏。其次，受到葡萄糖阻遏的碳源种类不同、酿酒酵母和不同非酿酒酵母的阻遏机制存在不同程度的差异，研究者需要根据实际需求决定将要采用的手段。最后，随着组学技术、第三代测序技术以及酵母单杂交、染色质免疫沉淀和 ATAC-seq 等转录因子与靶基因互作技术的发展，多种基因层面的分子操作日益丰富和完善，对于不同碳源受阻遏机制的研究方法以及缓解或解除碳代谢阻遏提出了新的见解和方法，为人类解决目前各领域实际生产问题提供更多的支持和可能性。

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