白念珠菌细胞内钙离子稳态和钙离子/钙调磷酸酯酶信号途径的调控机理研究进展

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摘要 钙离子稳态和钙离子/钙调磷酸酯酶信号途径在真核细胞中高度保守。与最简单的模式真核生物(酿酒酵母菌)一样, 人体病原真菌白念珠菌的细胞中存在各种钙通道、钙泵和钙交换器以及完整的钙离子/钙调磷酸酯酶信号途径成员, 它们在维持白念珠菌胞内钙离子稳态以及应答外界环境压力、耐药性、形态发生和致病性等方面有着至关重要的作用。对白念珠菌钙离子稳态和钙离子/钙调磷酸酯酶信号途径调控机理的认知, 有助于了解其致病过程和耐药机理, 同时可以为发现和开发新的抗真菌药物提供研究基础。该文结合所在实验室相关研究工作对这一领域的最新研究进展作了综述。

关键词 白念珠菌; 钙离子; 钙离子稳态; 信号途径

Progress in the Mechanisms Regulating Calcium Homeostasis and Calcium/Calcineurin Signaling in *Candida albicans*

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Abstract Calcium homeostasis and Ca^{2+}/calcineurin signaling pathway are highly conserved in eukaryotic cells. Like the simplest eukaryotic model organism, *Saccharomyces cerevisiae*, the human fungal pathogen *Candida albicans* has calcium channels, pumps and Ca^{2+}/H^{+} exhangers as well as components of the Ca^{2+}/calcineurin signaling, which play a vital role in maintaining the cytosolic calcium homeostasis and regulating the response to environmental stresses, the drug tolerance and the morphogenesis and virulence. Understanding mechanisms regulating calcium homeostasis and the Ca^{2+}/calcineurin signaling would help us to elucidate the pathogenesis and drug tolerance of *C. albicans*, and facilitate the discovery and development of new anti-fungal drugs. Here, based on our research work, we briefly summarize recent progress in this area of research.

Keywords *Candida albicans*; calcium ion; calcium homeostasis; signaling pathway

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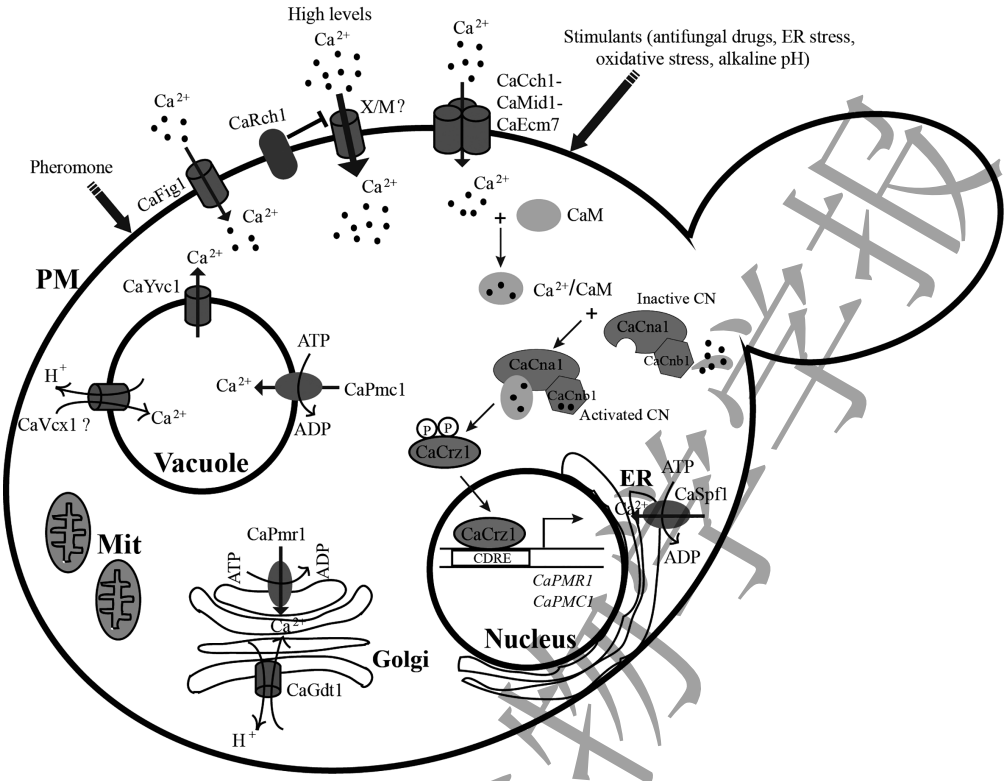
钙离子(Ca^{2+})作为细胞内信号分子调控细胞增殖、细胞程序性死亡、肌肉收缩和味觉[1-3]。在真核细胞中, 钙离子稳态和钙离子/钙调磷酸酯酶信号途径是高度保守的[4]。胞内钙稳态系统在真核模式生物——酿酒酵母中研究得较为详细。胞外高浓度钙离子条件下, 钙离子通过两个未知的质膜转运蛋白X和M进入细胞质中[5]。内质网胁迫、信息素、低渗胁迫以及低钙条件下, 由ScCch1、ScMid1和ScEcm7组成的位于质膜的高亲和性钙吸收系统是钙离子进入细胞的主要途径[6-8]。质膜上由ScFig1组成的低亲和性钙吸收系统参与细胞交配时的钙内流[9]。在低渗条件下, 液泡膜上的钙通道ScYvc1介导钙离子从液泡释放到胞质中[10]。然而, 胞质内钙离子浓度持续性地增加对细胞是有害的。胞质中过量的钙离子一方面通过液泡膜上的钙泵ScPmc1和Ca^{2+}/H^{+}交换器ScVcx1运输到液泡中[11-12], 另一方面通过高尔基体/内质网膜上的钙泵ScPmr1和Ca^{2+}/H^{+}交换器ScGdt1经蛋白分泌系统运输到胞外[13-15]。另外, 内质网膜上的离子转运蛋白ScSpf1也和钙离子稳态调控有关[16]。酿酒酵母细胞通过以上方式将胞质过多的钙离子运输到细胞器中以降低胞质中钙离子浓度, 使得胞质钙离子浓度维持在50~200 nmol/L范围内。胞质内钙离子结合钙调蛋白(calmodulin, CaM)后激活胞质内的钙调磷酸酯酶(calcineurin, CN), 钙调磷酸酯酶让胞质中的转录因子ScCrz1去磷酸化而导致它转移到细胞核内, 结合到靶标基因的启动子上, 调控包括*ScPMR1*和*ScPMC1*在内的靶标基因表达的上调[17]。钙离子/钙调磷酸酯酶信号途径在维持胞内钙离子稳态、应答外界压力以及维持细胞壁完整性等方面起到了重要的作用。

最近, 我们实验室通过功能基因组学手段从酿酒酵母基因组中筛选到共120个非必需基因, 它们的缺失导致酿酒酵母细胞对钙离子敏感, 其中包括7个蛋白质运输必需的内体分拣复合物(endosomal sorting complex required for transport, ESCRT)基因[18]。ESCRT复合物的功能是调节多囊体(multivesicular body, MVB)对靶向液泡(等同于哺乳动物细胞的溶酶体)的蛋白质的分拣过程[19]。我们对这7个ESCRT基因进行深入研究发现, *ScPMR1*除了受到钙离子/钙调磷酸酯酶信号途径的正向调控外, 还受到Rim101/Nrg1信号途径的负向调控[20]。ScNyv1是另外一个钙泵ScPmc1活性的负调节因子, ESCRT复合物可能通过调控*ScNYV1*基因的表达来控制ScPmc1的活性[21]。此外, 我们发现, 酿酒酵母细胞质膜上存在一个负向调控钙离子内流的新型调控因子, 该因子的转录表达本身却受到钙离子信号途径的正向调控[22]。

白念珠菌(*Candida albicans*)是临床上最常见的人体条件致病真菌[23]。存在于健康人体的黏膜表面, 包括口腔、消化道、阴道等处。在艾滋病患者、器官移植病人以及经过化疗和理疗的癌症病人等免疫能力低下的人群中, 白念珠菌可引发严重的系统性感染, 危及病人生命。由于在病人体内和植入体内的医疗器具表面形成生物膜、反复治疗过程中抗真菌药物的大量使用和预防性用药, 白念珠菌的耐药现象日益严重[24]。因此, 研究并揭示白念珠菌的致病与耐药机理、发现白念珠菌新型毒力因子以及开发新型抗真菌药物, 对白念珠菌感染的治疗具有重要意义。近年来的研究发现, 白念珠菌中存在与酿酒酵母相对应的钙通道、钙泵、钙交换器和钙离子/钙调磷酸酯酶信号途径成员(图1), 它们在维持白念珠菌胞内钙离子稳态以及应答外界环境压力、耐药性、形态发生和致病性等方面起着至关重要的作用[25-26]。本文结合所在实验室的相关研究工作, 对白念珠菌细胞内钙离子稳态调控的最新研究进展进行综述。

1 白念珠菌细胞质膜上的钙运输系统

与酿酒酵母[4]类似, 白念珠菌细胞质膜上钙内流系统由高亲和性钙吸收系统(high-affinity calcium uptake system, HACS)和低亲和性钙吸收系统(low-affinity calcium uptake system, LACS)组成。HACS系统由CaCch1、CaMid1和CaEcm7这3个成分组成(图1)[27-29]。CaCch1是哺乳动物电压门控Ca^{2+}通道(voltage-gated Ca^{2+} channels, VGCCs)α1亚基的同源蛋白, 而CaMid1是哺乳动物电压门控Ca^{2+}通道(voltage-gated Ca^{2+} channels, VGCCs)α2δ亚基的同源蛋白。ScEcm7是最近在酵母中发现的HACS的调节因子, 属于PMP-22/EMP/MP20/Claudin超家族跨膜蛋白质的一个成员, 是哺乳动物电压门控Ca^{2+}通道(voltage-gated Ca^{2+} channels, VGCCs)γ亚基的同源蛋



PM: 细胞质膜; CaM: 钙调蛋白; CN: 钙调磷酸酯酶; ER: 内质网; Mit: 线粒体; X/M: 两个未知的钙离子转运体; CDRE: 钙调磷酸酶依赖性元件; "┬"表示负调控。

PM: plasma membrane; CaM: calmodulin; CN: calcineurin; ER: endoplasmic reticulum; Mit: mitochondrion; X/M: uncharacterized calcium transporters X and M; CDRE: calcineurin-dependent response element; "┬": negative regulation.

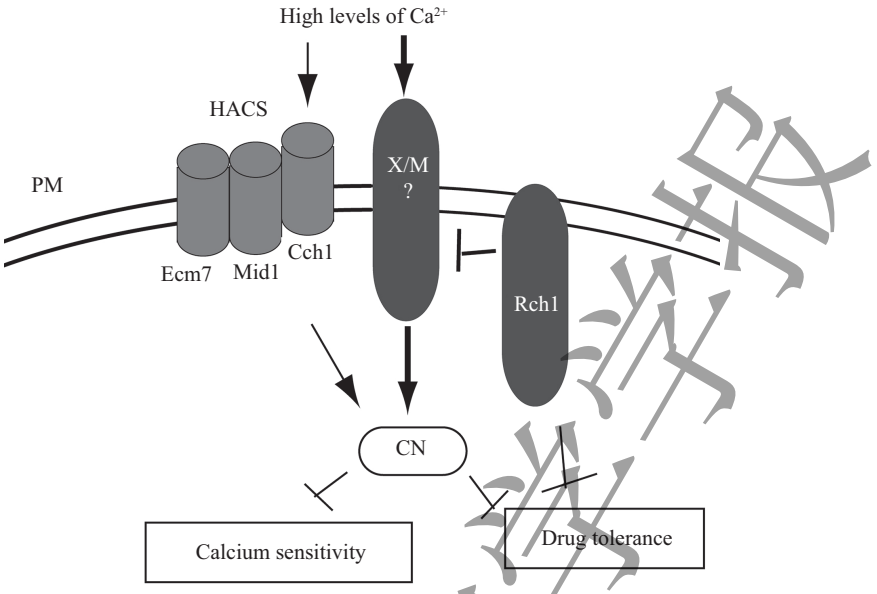
图1 白念珠菌细胞内的钙离子稳态系统和钙离子/钙调磷酸酯酶信号途径示意图

Fig.1 Calcium homeostasis and Ca^{2+}/calcineurin signaling pathway in *C. albicans*

白[8]。白念珠菌里的CaEcm7由514个氨基酸残基组成, 含有4个跨膜区。HACS是低钙、外界压力刺激条件下钙离子内流的主要通道。CaCch1、CaMid1或CaEcm7的缺失引起细胞钙内流减少, 导致细胞质内钙离子浓度降低, 引起细胞对抗真菌药物和氧化胁迫的敏感[27-33]。CaCch1或CaMid1的缺失导致细胞对N-糖基化抑制剂衣霉素敏感, 但是, CaEcm7的缺失则没有该作用。CaCch1或CaMid1的缺失造成细胞对低温敏感和菌丝定向能力降低[27,34]; 此外, HACS任何一种组分的缺失导致白念珠菌菌丝发育发生缺陷以及对小鼠毒力严重减弱[28-30,32-33]。因此, HACS在白念珠菌应答外界胁迫以及维持菌丝发育和毒力方面起着至关重要的作用。

细胞质膜的低亲和性钙吸收系统由CaFig1介导, 调节细胞交配条件下的钙离子内流[35]。CaFig1由265个氨基酸残基组成, 包含4个跨膜结构域, 与酿酒酵母ScFig1的同源性为48.5%, 具有PMP-22/EMP/MP20/Claudin超家族跨膜蛋白质的特征序列。CaFig1的缺失不影响低钙条件下钙离子的吸收和积累, 但显著影响菌丝的重新定向以及细胞交配[27,35]。

白念珠菌细胞质膜蛋白CaRch1是本实验室最近发现的新型钙稳态调节因子(图1)。CaRch1的缺失导致钙离子内流增加、胞内钙离子浓度增加以及钙离子/钙调磷酸酯酶信号途径的激活, 造成细胞对钙胁迫高度敏感[36]。因此, CaRch1是细胞外钙离子内流的负调控因子。CaRch1是人体第10个溶质转运蛋白家族第7个成员(solute carrier family 10 member 7, SLC10A7)的同源蛋白, 由411个氨基酸组成, 包含10个跨膜结构域, 第9个和第10个结构域的缺失不影响该蛋白质的功能和亚细胞定位[36]。本实验室最新研究发现, 敲除HACS任何一个组分(CaCch1、CaMid1或CaEcm7)均能够抑制*CaRCH1*缺失株的钙敏感性表型, 并且能够消除由于*CaRCH1*的缺失导致钙离子/钙调磷酸酯酶信号途径的激活[30,33]。此外,



PM: 细胞质膜; CN: 钙调磷酸酯酶; X/M: 两个未知的钙离子转运体; 符号箭头和"┬"分别表示正向和负向调控作用。
PM: plasma membrane; CN: calcineurin; X/M: uncharacterized calcium transporters X and M; Arrows and "┬": indicate positive and negative regulation respectively.

图2 白念珠菌细胞质膜上CaRch1和由CaCch1、CaMid1和CaEcm7组成的HACS系统之间相互作用, 调控钙离子敏感性和耐药性的模型

Fig.2 Working model illustrating genetic interactions between CaRch1 and the HACS system consisted of CaCch1, CaMid1 and CaEcm7 in calcium sensitivity and drug tolerance of *C. albicans* cells

敲除*CaRCH1*能够恢复由于*CaCCH1*或者*CaECM7*的缺失导致的菌丝发育缺陷。*CaRCH1*与HACS的3个基因之间的双基因缺失菌株对小鼠的毒力比这4个基因的每个单基因缺失株要高[33]。因此, CaRch1和HACS分别负向和正向调控调节白念珠菌胞内钙离子稳态, 从而共同影响其钙离子敏感性、药物耐受性以及菌丝发育和毒力(图2)。

2 白念珠菌内膜系统上的钙运输系统

2.1 高尔基体膜上Ca^{2+}/H^{+}交换体Gdt1的功能

真核生物内质网参与蛋白质的合成、修改与加工以及脂类合成与运输等过程。内质网中钙离子浓度约为10 μmol/L, 是胞质内钙离子浓度的100倍, 适度的钙浓度对维持内质网的正常功能是必需的[37]。高尔基体的主要功能是将内质网合成的蛋白质进行加工、分类与包装, 然后分门别类地送到细胞特定的部位或分泌到细胞外。此外, 蛋白质的糖基化起始于内质网, 完成于高尔基体[38]。内质网膜的离子转运蛋白CaSpf1(也称为CaCod1)属于P型ATPase, 由1 223个氨基酸残基组成, 具有10个跨膜区。CaSpf1的缺失导致细胞对缺钙条件、高钙条件、衣霉素以及抗真菌药物敏感, 同时导致细胞壁完整性途径、菌丝发育、生物膜形成及毒力的缺陷[39-40]。白念珠菌内质网/高尔基体膜上的钙泵CaPmr1也属于P型ATPase, 由917个氨基酸残基组成, 具有10个跨膜区。CaPmr1负责把胞质内的Ca^{2+}和Mn^{2+}转运至蛋白运输系统, 它的缺失导致细胞对缺钙条件敏感, 并且导致细胞壁组分蛋白N-糖基化和O-糖基化的严重缺陷以及白念珠菌毒力的丧失[41]。

CaGdt1是本实验室最近鉴定的位于白念珠菌高尔基体膜的Ca^{2+}/H^{+}交换体[34]。CaGdt1的缺失能够抑制由于CaCch1或CaMid1缺失所导致的低温敏感性和菌丝发育缺陷以及对抗真菌药物、衣霉素或荧光白化合物的敏感性。而且, *CaGDT1*和*CaMID1*双基因缺失株的毒力介于两个单基因缺失株之间。因此, 内质网/高尔基体内的钙离子稳态在白念珠菌的药物耐受性、细胞壁完整性、生物膜形成、菌丝发育和毒力等方面起着重要的作用。

2.2 液泡膜

真核生物液泡的功能是多方面的, 包括细胞组

分的降解与周转、离子和代谢物的储存、养分运输和调节细胞生长等[42]。液泡是酿酒酵母细胞的主要钙库, 细胞中90%的钙离子储存在液泡中[12]。CaPmc1是位于白念珠菌液泡膜上P型ATPase, 负责把胞质内的钙离子泵到液泡内。CaPmc1的缺失导致细胞对钙离子胁迫极度敏感以及对抗真菌药物耐受性增加[36,43]。CaYvc1是位于白念珠菌液泡膜上的钙离子通道, 介导液泡内钙离子向胞质释放, 属于瞬时受体电位(transient receptor potential, TRP)通道蛋白家族的成员。CaYvc1的缺失影响胞内钙离子稳态、细胞膜压力、菌丝发育、极性生长、生物膜形成以及毒力等[44]。

3 白念珠菌细胞中钙离子/钙调磷酸酯酶信号途径

真核细胞中钙离子/钙调磷酸酯酶(Ca^{2+}/calcineurin)信号途径是高度保守的[45-46]。与酿酒酵母细胞[1-2,4]一样, 白念珠菌细胞中存在由钙调蛋白、钙调磷酸酯酶和转录因子CaCrz1组成的完整信号系统[25-26]。白念珠菌的钙调蛋白序列和结构与酿酒酵母高度保守[47]。此外, 钙调蛋白特异性抑制剂N-(6-aminohexyl)-5-chloro-1-naphthalene-sulfonamide和Trifluoperazine可以抑制白念珠菌的菌丝发育[48-49]。

钙调磷酸酯酶属于丝氨酸/苏氨酸蛋白磷酸酯酶家族成员, 它的结构和功能在包括酵母和哺乳动物在内的真核细胞中是高度保守的[50]。它以异二聚体的形式存在, 由催化亚基A和调节亚基B按比例1:1组成。催化亚基CaCna1或者调节亚基CaCnb1的缺失都可以导致白念珠菌细胞对细胞质膜胁迫[十二烷基硫酸钠(sodium dodecyl sulfate, SDS)]、细胞壁胁迫(荧光白、刚果红)、抗真菌药物、离子胁迫(Ca^{2+}、Li^{+}、Na^{+}或Mn^{2+})、高酸性环境(pH2)和内质网胁迫(衣霉素)的敏感以及在血清中的存活能力和对小鼠的毒力下降[51-53]。最近研究表明, 作为分子伴侣的热休克蛋白90(heat shock protein 90, Hsp90)可以与钙调磷酸酯酶直接结合, Hsp90介导的棘白菌素抗性是通过钙调磷酸酯酶起作用的[54]。*CaRCH1*缺失株的钙敏感表型可以被钙调磷酸酯酶特异性抑制剂CsA抑制, 进一步说明了CaRch1缺失引起的钙敏感表型是通过钙调磷酸酯酶起作用的[33,36]。

白念珠菌转录因子CaCrz1是钙调磷酸酯酶的靶蛋白[55]。CaCrz1能识别和结合靶基因启动子里的CDRE序列[5′-G(C/T)GGT-3′], 从而调控它们的表达。白念珠菌中有60个基因的表达上调依赖于钙调磷酸酯酶和CaCrz1, 这60个基因与离子运输和稳态、压力应答、细胞壁组装和合成、细胞骨架组装和合成、碳源代谢、细胞周期和磷脂合成等功能有关[55]。*CaCRZ1*缺失株对Ca^{2+}、Li^{+}、Mn^{2+}、SDS、抗真菌药物的敏感性低于*CaCNA1*缺失株, 并且*CaCNA1*缺失株对小鼠毒力完全丧失, 而*CaCRZ1*缺失株仅丧失部分毒力[43,55]。因此, CaCrz1不是钙调磷酸酯酶唯一的靶蛋白, 钙调磷酸酯酶的其他靶蛋白有待于进一步的鉴定和研究。

4 总结与展望

近年来, 白念珠菌钙离子稳态和钙离子/钙调磷酸酯酶信号途径的研究取得了长足的进展, 酿酒酵母系统中许多组分的白念珠菌同功物已经被鉴定(图1), 而且它们在白念珠菌细胞生长、菌丝发育和毒力方面的功能也得到较为详尽的阐明。然而, 还有众多问题有待解答。与酿酒酵母液泡Ca^{2+}/H^{+}交换体ScVcx1同源的白念珠菌CaVcx1的功能还有待进一步研究。酿酒酵母细胞中存在2个尚未鉴定的质膜钙离子转运蛋白X和M, 它们也可能存在于白念珠菌细胞中。我们实验室发现的质膜CaRch1蛋白的靶蛋白还未得到鉴定, 这个问题的阐明将有助于对这一蛋白质负向调控胞内钙离子稳态机制的了解。最近的研究表明, ESCRT复合物对白念珠菌的致病性非常重要[56]。我们实验室的研究结果表明, 酿酒酵母中, ESCRT调控钙泵基因*ScPMR1*的表达[20], 那么ESCRT是否通过调控胞内钙离子稳态来影响白念珠菌的致病性呢? 此外, 转录因子CaCrz1进入细胞核及输出细胞核的机制, 钙调磷酸酯酶其他靶蛋白的发现和功能鉴定, 这些科学问题的回答将有助于全面了解钙离子和钙调磷酸酯酶信号途径的调控机理, 同时有可能提供新的抗真菌药物靶标, 为真菌感染的防治提供新的策略。

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